CHARACTERIZATION OF LENTIL GERMPLASM THROUGH MORPHOLOGICAL AND METRIC TRAITS AND MOLECULAR MARKERS

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By
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CERTIFICATE

This is to certify that the thesis entitled **"Characterization of lentil germplasm through morphological and metric traits and molecular markers"** submitted to the **Bundelkhand University, Jhansi** for the award of Degree of **Doctor of Philosophy in Botany (Genetics and Plant Breeding)** is a record of *bonafide* research work carried-out by **Miss Poonam Singh** under our guidance and supervision.

This thesis embodies the work of the candidate herself. The candidate worked under us from the date of starting the research work. She has put in 220 days attendance.

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LIST OF ABBREVIATIONS

AFLP	:	Amplified fragment length polymorphism
cDNA	:	Complementary DNA
cM	:	Centi-morgan
cTAB	:	Hexadecyl trimethyl ammonium bromide, cetrimide
dATP	:	Deoxyadenine triposphate
dCTP	:	Deoxycytosine triposphate
dGTP	:	Deoxyguanine triposphate
DNA	:	Deoxyribonucleic acid
dNTP	:	Deoxyribonucleotide triphosphate
dTTP	:	Deoxythiamine triphosphate
EDTA	:	Ethylene diamine tetra acetic acid
Est	:	Esterase
F	:	Filial generation
Gity	:	Griffty
Inter-SSR PCR	:	Inter- simple sequence repeat polymerase chain reaction
Ml	:	Millilitre
mM	:	Millimolar
N_2	:	Nitrogen
NaCl	:	Sodium chloride
Ng	:	Nanogram
NOR	:	Nucleolar organizer region
PCA	:	Principal component analysis
PCR	:	Polymerase chain reaction
QTL	:	Quantitative trait loci
RAPD	:	Random amplification of polymorphic DNA
RFLP	:	Rrestriction fragment length polymorphism.
RNase	:	Ribonuclease
rpm	:	revolution per minute
SCAR	:	Sequence characterized amplified region
SDS-PAGE	:	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

SI	:	Similarity index.
TAE	:	Tris-acetate EDTA buffer
TE	:	Tris- EDTA
Tpi-p	:	Triosephosphate isomerase
UPGMA	:	Unweighted pair group method on arithmetic average
µl	:	microlitre
µM	:	Micro-molar

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important food legume crop of the Indian subcontinent, the Middle-East, North Africa and North America. It is proposed to be originated in the fertile crescent of the Near–East and cultivated at the beginning of agriculture itself. The remains of lentil seeds have been found in archaeological sites throughout the Middle-East indicating that Neolithic man was already familiar with this plant during 8000B.C. (Renfrew, 1969). The valleys in the Hindukush Mountains in India are also claimed as its native place.

According to Williams *et al.*, (1974) lentil *(Lens culinaris* Medik.) belongs to the order Rosales, suborder Rosineae, family Leguminoceae, sub-family Papilionaceae and genus *Lens*. The Latin word *"Lens"* describes exactly the shape of seed. The Latin name of lentil, *Lens culinaris,* was first published by Medikus in 1787. According to Barulina (1930), the species has been divided into two main groups: subspecies *macrosperma* (Baumg. provar.) Barulina which produces large pods (15.20x7.5-10.5mm^2), with more flattened seeds (100-seed weight 9.0-8.0g), usually with yellow cotyledon and pale green testa. The second subspecies *microperma* (Baumg. provar.) Barulina, is characterized by small pods (6.15x3.5-7mm^2), with small round seeds (100-seed wt. 2.0-4.0g), yellow or orange cotyledons and testa of various colours, ranging from pale yellow to black. The *microsperma* group is generally considered to be elder than *macrosperma* group and is mainly cultivated in the Indian subcontinent.

Based on breeding experiments and cytogenetic analysis of F_1 hybrids, Ladizinsky (1979a) concluded that *Lens orientalis* is the wild

progenitor of the cultivated lentil. However, all the *Lens* species are diploid with chromosome number 2n=14. The phylogenetic relationship among subspecies in the genus *Lens* was investigated based on morphological, biochemical (SDS-PAGE) and molecular markers (RFLP and RAPD) which revealed that *Lens culinaris* ssp *orientalis* was the progenitor of cultivated lentil (Ahmed and Mc Neil, 1996).

Among pulse crops, lentil is next to *Lathyrus* in its ability to sustain under moisture stress conditions. It has the potential to cover the risk of dry land agriculture and can be used as a cover crop to check soil erosion in problem areas. Lentil also fixes atmospheric nitrogen through nodules in roots in association with *Rhizobium* sp., which increases the fertility status of soil. The lentil crop can fix atmospheric nitrogen up to 107 Kg/ha (Verma *et al.*, 1991).

Lentil is also valued as a high protein food for its nutritive constituents including straws, which are used in cattle feeding. Lentil is mostly used as *"dal"*. its splitted cotyledons are normally orange or yellow in colour. its whole grain are also used in different dishes and culinary purposes. Besides high yield potential, its inbuilt capabilities to sustain moisture stress and with stand problematic soil conditions imparted a very wide adaptability to this crop. In addition, lentil has a distinct export potential among the pulses of Indian origin. Although lentil has been an important food legume but little attention has been paid on genetic studies in lentil until recently. The conventional breeding methods successfully operate on existing genetic variability/diversity available in the germplasm/base population. This variability may also be created artificially and then playing on it through various plant breeding methods in later generation.

Lentil is an annual herb, green in colour, freely branches with slender stem and soft hairy foliage with average plant height of 25-40 cm. Leaves are pinnately terminated by a bistle or tendril, stipules linear, leaflets opposite or alternate. Flower 1-4 in axillary inflorescence, Peduncles slender, flower small upto 8mm long, calyx narrowly and lobed, corolla pale blue white or pink, diadelphous stamen (9+1). Pods oblong smooth, about 1.3cm long containing 1-2 seeds. The seeds are lens shape. The germination of lentil is always hypogeal (Yadav, 1991).

Since lentil is a winter season crop, it can be grown after harvest of any *kharif* crop. The important crop rotations are rice-lentil, rice-lentil-green manuring and maize/pearl millet/sorghum-lentil etc. It is also gaining popularity as an *utera* crop especially in Bihar where lentil seeds are broad casted before the harvesting of rice.

It is mostly grown in the northern plains, central and eastern parts of India. The states like Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal are the major lentil growing areas in India. The total production of 900 thousand metric tones which cover the area of 1100 thousand hectare and average yield 818 Kg/h have been recorded in lentil (FAO, 2004).

The only way to make this crop competitive to other crop is its genetic up gradation in respect of resistance to diseases and insect pest, yield potential and nutritional qualities. The genetic up gradation in only possible when the genetics of traits/ problems is known to the breeders and geneticists. Having generated genetic information on these traits/problems, there are two probable routes through which genetic up-gradation could be effected in this crop, i.e. the simply inherited traits and simple problems can be tackled by conventional breeding methods,

whereas for the improvement of complex traits and difficult problems the help of biotechnological tools can be sought.

The genetic divergence among the germplasm accession and base populations is a pre-requisite for making selection of the parents for effective hybridization. It is a fact that greater the divergence among the parents, more are the chances of generating array of variability in the population of their crosses. Mahalanobi's D^2 statistics is a useful and widely used parameter of assessing genetic divergence in the germplasm.

Among the selection parameters, heritability and genetic advance are quite useful for laundering crop improvement programmes successfully. Where the farmer speaks about the magnitude of genetic variability which is transmissible from parents to their off-springs, on the other hand, the genetic advance assesses/predicts the grain to be effected in the progeny of the selected individuals one generation in advance. There are two ways of estimating heritability, i.e. in broad sense, which is the proportion of genetic variability to total variability is worked out. The estimate of heritability in narrow sense is more useful and reliable as it indicates the extent of heritability which helps the breeders in selecting/rejecting the population, depending upon the magnitude of the estimate. Also, lower the estimate, poor are the chance for effecting improvement and hence rejection of the population is desirable in such situations and vice-versa. Also, on the basis of the magnitude of this estimate after the acceptable limit, the intensity of the selection may be decided, i.e. higher the value lower would be required the intensity of selection and vice-versa.

The knowledge of correlation between two or more than two characters is helpful in exercising selection. For example, if two desirable

characters are positively correlated, in the situation of the selection of only one trait the other trait gets automatically selected. However, in the situation where one desirable character is positively associated with undesirable one, the breakage of this association through intensive crossing/inter mating exercise is required. Therefore, before starting selection programme, the knowledge about the nature and magnitude of correlation among the characters under consideration is essential. Similarly the knowledge as how the most important dependable character like yield or any economic trait is favorably influenced by its component traits, whether directly or via indirect contributors, is essential. This information could be derived through path analysis of the data recorded on dependent and its individual component traits.

The germplasm in any crop is the genetic wealth of that country where it is inhabited/stored/conserved. Therefore, all activities related to collection, evaluation, maintenance, characterization, cataloguing, utilization and conservation of germplasm are important for preserving and exploiting the variability. Among these activities, characterization of germplasm is the most important one for cataloging so that immediate users can easily pick up the desired line(s) based on one or more desirable trait in which they are interested.

The characterization and cataloguing of germplasm have been carried out in the past using mostly morpho-agronomic markers. But these morphological markers are oftenly affected by environment and growing practices and most of them are visible at a particular stage of plant growth and development. These markers, though indicative of the genotype, are represented by a few loci, because there are not in large enough number of in relation to number of genotypes in germplasm. However, advances in molecular biology have introduced as complementary

approach for germplasm characterization and classification. Biochemical and molecular markers, which are being used in genetic characterization of the germplasm in a number of crops, is a recent innovation. These markers are powerful tools that can yield significant information that may enhance the scope of use of germplasm in the crop improvement programme. The genetic characterization of germplasm also helps in their effective conservation and reveals the extent of relationship among the accession as they estimate diversity. The isozymes analysis (biochemical marker) is used to estimate the extent of variability present in plant population, but has certain limitations due to the availability of only a limited number of marker loci, the general lack of polymorphism for these loci in breeding materials. With the advent of DNA markers (Paterson *et al.*, 1991), a reliable identification system of germplasm can be developed. Different molecular marker like restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), amplified fragment length polymorphism. (AFLP) and randomly amplified polymorphic DNA (RAPD) have been widely used for estimate genetic diversity and tagging of traits. In this context, randomly amplified polymorphism DNA (RAPD) is a reliable technique for genotype identification and characterization. However, RFLP analysis is laborious and time consuming, AFLP analysis is costly and specialization is needed, whereas SSR analysis requires crops specific primers. The RAPD analysis on the other hand has several advantages over other molecular markers:

a) It is comparatively easy, cost effective and minimum time required.

b) No clones or DNA sequence information is needed.

c) Sets of several hundred primers available commercially.

d) Multitude of potential primer binding site throughout the genome.

Therefore, the molecular analysis based on RAPD markers was performed in the present study.

In view of the importance of lentils as human diet, animal feed and fodder and cropping system and the problems, the present investigation **"Characterization of lentil germplasm through morphological and metric traits and molecular markers"** has been planned with the following major objectives:

1. To classify germplasm lines on the basis of morphological markers.
2. To Estimate variability in the germplasm lines.
3. To estimate genetic divergence among the lines.
4. To estimate correlation among important economic characters.
5. To work out direct and indirect effects of different traits on yield through path analysis.
6. To estimate heritability and genetic advance.
7. Exploitation of RAPD markers in germplasm characterization diversity analysis.

REVIEW OF LITERATURE

Lentil is an ideal plant for basic genetic research as it is sexually propagated, amenable for emasculation and pollination, limited number of distinct chromosomes etc. Unfortunately, this crop could not get as much attention of the geneticists and breeders may be due to its consideration as minor pulse crop. This is the reason that genetic information, as is available in many cereals, vegetable, oil seeds and even in some legumes, required for planned breeding programme, is not available in this crop. However, whatever information available has been reviewed under the following heads, which are related to the present study:

2.1. Characterization based on morphological markers

2.2. Variability

2.3. Genetic divergence

2.4. Correlation

2.5. Path coefficient analysis

2.6. Heritability

2.7. Genetic advance

2.8. Molecular markers

2.1. Characterization based on morphological markers

A. Inheritance of qualitative traits

1. Growth habit

Ladizinsky (1979b) found three different types of growth habit (erect, intermediate and prostrated) in an F_2 population of different

crosses between erect and prostrate types and assigned gene symbol *gh gh* for spreading type *Gh Gh* and for tall type. Emami (1996) reported that spreading type was dominant over erect growth habit with monogenic control based on analysis of seven crosses. He proposed the spreading type of growth habit due to the dominant allele *Ert* and the erect phenotype appears in homozygous recessive *ert ert.*

2. Pubescence

The inheritance of presence and absence of pubescence on peduncle was studied in six crosses involving pubescent and non pubescent (glabrous) peduncle portents. The results revealed that all the F_1 plant showed pubescent character and their F_2 population were well fit with 3 pubescent: 1 non pubescent ratio indicating the monogenic dominance of pubescent over non-pubescent peduncles. The gene symbol *Pdp* has been proposed for pubescent peduncle, *pdp, pdp,* being glaborous. Here *pd* stands for peduncle and *P* for pubescence (Emami 1996). Hoque *et al.*, (2002) concluded the dominance of pubescent development on plant over glabrous. The glabrous plant is caused by a single recessive gene in homozygous condition. They proposed gene symbol *Pub Pub* for pubescent plant and *pub pub* for glabrous phenotype.

Vandenberg and Slinkard (1989) reported dominance of pubescent pod over glaborous ones. A single recessive gene in homozygous condition, therefore, causes the glaborous pod. They proposed gene symbol *Glp Glp* for pubescent pod and *glp glp* for glaborous pod.

3. Pigmentation

(i) Brown leaf pigmentation

Emami and Sharma (1998) studied the brown pigmentation of leaf in 11 crosses involving a genotype with pigmented and 4 with non pigmented (green) leaves. The analysis of 4468 F_2 plants under field conditions revealed perfect monogenic segregation into 3 brown : 1 green ratio. The gene symbol *Bl* (brown leaf) has been proposed for this trait and homozygous recessive state *bl bl* expresses green leaf phenotypes

(ii) Stem pigmentation

Ladizinsky (1979) reported that pigmentation stem was completely dominant over green stem. He proposed gene symbol *Gs* for this trait, where *gs gs* plants do not produce any pigment in their stem and remain green all through their life. The same results have also been reported by Emami (1996).

4. Tendril formation

Tendril formation at the leaf apex of lentil was reported to be controlled by a single dominant gene (Vandenberg and Slinkard, 1989). They proposed the gene symbol *Tnl* the to represent this trait. Therefore, homozygous tendrilled plants have the genotype *Tnl Tnl* and tendrilless phenotypes appear only under homozygous recessive condition *(Tnl Tnl).*

5. Leaf colour

Mishra *et al.* (2001) reported that the dominance of dark green foliage over light green foliage. They proposed gene symbol *Dgl Dgl* for dark green foliage and *dgl dgl* for light green phenotype. Haque *et al.*

(2002) obtained similar result and used same gene symbol *(Dgl)* as reported by Mishra *et al.* (2001) for dark green foliage.

6. Flower colour

This trait is extremely variable in lentil and poses difficulty in screening. The standard colours can be white, bluish, violet and even pink. The colour of the keel and wings is sometimes lighter or darker than the background of standard, thus forming various combination of flower colour.

Lal and Srivastava (1975) reported F_2 segregation into 3 violet : 1 white and 3 violet : 1 pink in the crosses between parents with violet × white and violet × pink flowers, respectively. Crosses between white × pink flowered genotype yielded four phenotypes in the F_2 i.e. violet, pink, white and rose, in the ratio of 9:3:3:1. This suggests two-gene control of this trait with complete dominance without epistasis. The gene symbol *V* was proposed for violet and *p* for pink and thus, the genotype, for the four classes are *V-P-* (violet), *VVpp* (white), *vvPP* (Pink) and *vvpp* (Rose).

Wilson and Hudson (1978) reported the F_2 ratio of 9 violet : 6 intermediate : 1 white, suggesting additive effect of two genes. They found that white flower might have pale-pink or pale violet veins deep white in the throat of the standard petal. They proposed gene symbols *V* for violet and *W* for white, with the genotypes *VVWW* violet, *VVww* and *vvWW* intermediate and *vvww* white.

Gill and Malhotra (1980) confirmed the F_2 ratio of 3 violet : 1 white flowered plants in lentil. Ladizinsky (1970) also reported 3:1 ratio on an F_2 generation of a cross between blue × white flowered lentils. Since the blue flower colour was not included in the previous studies (Lal & Srivastva, 1975; Wilson and Hudson, 1978), it can be assumed that at

least three genes are involved in the development of flower colour in lentil.

7. Testa colour

Inheritance of this trait seems to be fairly complicated. Different segregation ratios were reported by different lentil workers. Wilson and Hudson (1978) crossed lentil genotypes with black and beige seed coats. They assumed existence of two factors without dominance based on the ratio of 1 black : 14 mottled : 1 beige for this character in F_2 generation.

Vandenberg and Slinkard (1990) studied F_2 generation of crosses between lentil strains with brown and green seed coat, and observed four classes of testa colour; brown, gray, tan and green. Based on this, authors recorded segregation in the 9:3:3:1 phenotypic ratio and concluded that the ground colour of seed coat in lentil is controlled by two dominant genes at non-linked loci. The gray ground colour is determined by the dominant gene *Ggc,* and tan ground colour is determined by the dominant gene *Tgc.* They proposed the genoptypic combinations for different phenotypes as follows: *Ggc Ggc Tgc* (brown), *Ggc, Ggc, Tgc, Tgc* (gray), *ggc,ggc,Tgc,*(tan) and *ggc ggc tgc tgc* (green).

Valliancourt and Slinkard (1992) reported that the seed coat colour was determined by a single gene with black seed coat dominant over non-black. They proposed gene symbol *Blsc* for this trait. Emami(1996), however, contested the model of gentic control of black testa in lentil proposed by the earlier workers. He showed that although black testa is dominant over non-black testa (Emami and Sharma, 2000) and proposed gene symbol *Blt* for this trait.

Singh and Singh (1993) reported a ratio of 3 brown: 1gray mottle in the F_2 generation of crosses between parents with brown and gray

mottled seed coat. They proposed gene symbol *B* for brown and *b* for gray mottled seed coat colour. However, with the detailed analysis of black testa colour by (Emami and Sharma, 2000) the understanding of genetic control of black testa colour has changed substantially. This trait is designated by gene symbol *Blt* and the gene symbol *B* has been assigned to brown (Brownish yellow) cotyledon colour (Emami and Sharma, 1996b).

Analysis of the F_2 populations of crosses between strains bearing seeds with brown and tan testas revealed monogenic segregation with dominance of brown over tan seed coat colour (Emami, 1996).

The inheritance of cotyledon and testa colours in lentil were studied by Emami (1996) in seven crosses using parents with black, brown, tan and green testa in combination with orange, yellow or dark green cotyledon. Analysis of F_2 and F_3 seeds revealed that although black testa is dominant over non-black testa, its penetrance is not complete since both F_1 plants and heterozygous F_2 plants produced varying proportions of seeds with either black or non-black testa. The F_2 population of the crosses between parents with brown and tan, as well as brown and green testa segregated in the ratio of 3 brown: 1 tan and 3 brown: 1 green, respectively, indicating monogenic dominance of brown testa colour over tan or green. The expression of testa colour was influenced by cotyledon colour when parents with brown and green testa were crossed with those having orange or green cotyledons. Thus, F_2 seeds with green testa always had green cotyledons and never orange cotyledons. The F_2 seeds with brown testa always had orange cotyledon. These results suggest diffusion of a soluble pigment from the cotyledons to the testa (Emami and Sharma., 2000).

8. Seed coat pattern or testa pattern

Ladizinsky (1979B) obtained 3:1 ratio of plants with spotted and spotless seed coat and proposed the gene symbol *Scp* for seed coat pattern, with the spotless genotype written as *scp*. Based on a series of crosses involving parental lines with different seed coat patterns, Vandenberg and Slinkard (1990) concluded that there is a series of multiple alleles at the *scp* locus. Marbled –1 *(Scpm-1)* is dominant over absence of pattern *(scp)*. The second allele of series *scpm-2*, is dominant over *scps, scpd, scp*. The *scps* and *scpd* allele are co-dominant, and both are dominant over *scp* (patternless). Vandenberg and Slinkard (1990) also studied several other crosses involving different seed coat patterns. Crosses between genotypes with dotted testa in combination with flecks (DF) and dotted without flecks produced F_2 populations which segregated in to 3DF: 1 dotted seed coat patterns, which suggests that DF should also be included in the multiple allelic series at the *scp* locus. However, they could not determine the dominant-recessive relationship of DF with the other *scp* alleles reported by them.

Inheritance of seed coat pattern studied by Emami (1996) revealed complete dominance of the presence of any kind of pattern, such as mottling, speckling or their combination over is absence. Emami (1996) distinguished only two types of seed coat patterns; one, comprising minute dot like spots spread uniformly all over testa was called mottling. The other pattern, called speckling, was made of large irregularly shaped patches of colour and distributed haphazardly at variable distance from each other. When a cross was made between mottled and speckled seeded plants, the F_1 phenotype was a mixture of both the patterns (Combined). The F_2 plants segregated into 1 mottled : 2 combined : 1 speckled, indicating co-dominant behavior of the two testa patterns. Since some of

the F_2 plants with combined phenotype were true breeding, it was concluded that the two types of seed coat pattern, i.e. mottling and speckling, are controlled by two different but linked genes. The gene symbols *Mot* and *Spt* was proposed for mottling and speckling testa, respectively (Emami, 1996).

9. Cotyledon colour

The first report on genetics of lentil was based on the study of inheritance of cotyledon colour (Tschermark-sey senegg, 1928; Wilson *et al.,* 1970) reported that the F_1 seed from reciprocal crosses between orange (also called red) and yellow cotyledon parents was red indicating complete dominance of the orange cotyledon colour. The F_2 seeds segregated in the 3 red : 1 yellow ratio indicating that the trait is determined by a single dominant gene.

The inheritance of cotyledon colour was also studied by Singh (1978), Slinkard (1978), Sinha *et al.,* (1987). Singh (1978) referred to the red cotyledon as orange and orange proposed the gene symbol 'O' for orange and 'o' for yellow. Slinkard (1978) also presented data on the inheritance of green cotyledon in lentil and concluded that the red cotyledon character was completely dominant over green. In the F_2 generation of a cross between red and some green cotyledon parents, he observded the ratio 9 red : 3 yellow: 4 green, indicating digenic segregation with the involvement of a recessive colour inhibitor gene. The following gene symbols were proposed:*Yc* (orange-cotyledon), *yc* (yellow-cotyledon), and i-*yc* (inhibitor of the cotyledon colour producing green cotyledons). i-*yc* does not inhibit the expression of either *Yc* or *yc* alleles in its dominant state. The genotype of homozygous green cotyledon plants would be either *yc-yc,* i-*yc-i-yc* or *Yc Yc i-yc i-yc.* In

other words, orange or yellow cotyledons are produced only if the *i-Yc* gene is dominant or recessive codominant e.g *i-yc i-yc. YcYcYc* (Orange) and *i-yc i-yc yc* (Yellow).

The inheritance of cotyledon colour was investigated more precisely by Emami (1996) in the crosses involving parents with all possible variations. He recognized two basic colours: bright yellow (called yellow) and brownish yellow (called brown) which were designated by the gene symbols *Y* and *B*, respectively. The orange colour was found to be monogenically dominant over yellow and brown. The crosses between parents with orange and light green cotyledons segregated into four phenotypic classes in the F_2 i.e. orange, yellow, brown and light green in the ratio of 9:3:3:1. This strongly suggested the involvement of two independent gene controlling cotyledon colour (Emami and Sharma, 1996b). According to this hypothesis the four phenotypic classes would be expected to have the following gene combination. *Y-B-* (orange), *Y-bb* (yellow), *yyB-*(Brown) and *yybb* -(light green).

Another gene was discovered by Emami (1996), which leads to production of dark green cotyledons in recessive condition. This single gene, when mutated, eliminates both the yellow and brown pigments in the cotyledons. This third gene was called *Dg* (for dark green cotyledons). It has been hypothesized that the gene *Dg* operates at on earlier stage in the pathway of pigment synthesis and in recessive condition *(dg dg)* block the synthesis of the two pigments which in combination cause orange coloration of the cotyledons. It has been suggested that the *Dg* gene is involved in the synthesis of a common precursor or of both the yellow and brownish yellow pigments (Sharma and Emami, 2002). In other words, yellow or brown pigments express only when the *Dg* gene is dominant and functional. Depending on the

status of the *Y* and *B* genes the gene combinations responsible for different phenotypes are *Dg-Y-B-* (orange), *Dg-Y-bb-* (yellow), *Dg-yy B-* (brown) and *Dg -yybb* (light green). In recessive condition *(dg dg)* only dark green colour express irrespective of whether the genes *Y* and for *B* are in dominant or in recessive condition.

10. Number of flowers per peduncle

Gill and Malhotra (1980) studied the inheritance of number of flower per inflorescence. They crossed a lentil strain with two flowers per raceme with a genotype developing three flowers per peduncle and observed F_2 segregation in the ratio of 3 two flowered : 1 three flowered plants. They proposed gene symbol *Fn* for two-flowered and *fn* for three-flowered, where *Fn* stands for flower number.

Emami (1996) reported that the number of flowers per peduncle usually varies from 2-4 in lentils. He made nine crosses between genotypes with variable number of flowers per peduncle to study the inheritance pattern of this trait. The F_2 segregation did not show any specific pattern in majority of crosses. However, in two crosses involving two flowered and three-flowered parents good fit to the 3:1 three-flowered:two-flowered ratio was obtained.

11. Days to flowering

In F_2 populations of 25 crosses (early × early, early × medium, early × late, medium × medium, medium × late and reciprocal crosses in some of these combinations were studied by Emami (1996). He proposed the transgressive segregation in F_2 populations of the crosses between early × early and early × medium parents and the range of flowering was 45-120 days. In the crosses involving late × late parents, the range was

100-120 days to flowering in F_2 generation. A few F_2 plants in these crosses did not flower even after 125 days and the plants were forced to dry due to high temperature.

B. Inheritance of quantitative traits

Quantitative traits are one of the important parameters for determining the efficient breeding programme. The traits like plant height, days to flowering and maturity, number of pods/ plant, seed weight, biomass score, grain yield, harvest index etc are important quantitative characters which have direct effect on the yield. Different workers observed considerable heterosis in respect to quantitative traits. Some of these have been reviewed in present context.

Singh and Jain (1971), Sagar and Chandra (1980), Sandhu *et al.* (1981), Bhajan *et al.* (1987) and Tyagi and Sharma (1989) observed heterosis for number of branches/plant, pods/plant and cluster/plant, harvest index and earliness in flowering and maturity, indicating non-additive genetic variance for these traits. The predominance of non-additive genetic variance for seed yield/plant have also been reported by Singh and Jain (1971), Chauhan and Singh (1995). The grater importance of additive genetic system in the inheritance of 100-seed weight has been reported by Singh and Singh (1993) and Chauhan and Singh (1995). The importance of both additive and non-additive genetic variance for branches/plant has been reported Sagar and Chandra (1980) and Waldia and Chhabra (1989). High estimates of heritability were obtained for yield/plant, 100-seed weight, plant height, days to flowering, germination percentage, total plant weight (biomass) and maturity. Nandan and Pandaya (1980), Lakhani *et al.* (1986) and Singh *et al.* (1989) recorded low estimates of heritability for grain yield, seed size, seed/pod,

pods/plant and number of primary braches (Sindhu and Mishra, 1982; Ramgiry *et al.*, 1989 and Singh *et al.*, 1989).

2.2 Variability

Variability is the hub of any breeding programme. The success of breeding programme mainly depends upon the nature and magnitude of variability. Higher the variability, greater are chances of attaining better success in the selection and vice-versa. Also, among the three components of genetic variability, i.e. additive, dominance and epistemic, additive one, which is fixable, is of great importance in selection breeding. Therefore, before launching any breeding programme, the knowledge about the magnitude and kind of the genetic variability in the germplasm is essential. The literature on variability for different characters in lentil has been reviewed as follows:

Rajput *et al.* (1989) reported twenty two lentil genotypes of diverse origin were sown at one site to measure genotypic and phenotypic variances and the association of yield with other quantitative traits like maximum plant height (52.4cms.) and highest number of seeds/plant. Genotypic and phenotypic variances were very high for number of pods/plant, followed by plant height.

Rajput *et al.* (1989) reported information on genetic variance and yield correlation is derived from data on yield and six related traits in 22 accessions of diverse origin grown at one site.

El.Attar.A.H. (1991) reported twenty Germplasm lines from the ICARDA Lentil collection. All the five characters like plant height, 100-seed with seed yield, biomass score and harvest index studied were significantly different among the germplasm, except number of seed /pod.

Ismail *et al.* (1994) reported information on genetic variation is derived from data on six yield components and seed protein content in the F_3, F_4 and F_5 generations of 4 lentil crosses made between five local and exotic genotype grown during 1988-89.

Kumar *et al.* (1995) reported information on genetic variability is derived from data on 10 yield components in 432 *culinaris* (3 male and 10 female parents and their 30 F_1 hybrid genotypes grown at Faizabad, Uttar- Pradesh during *rabi* (1991-1992).

Chauhan *et al.* (1998) reported genetic variability was estimated for seed yield and 15 other characters in lentil (*Lens culinaris* Medik) experimental material included six parental lines and 15 F_1s during the 1992-94-winter season. Maximum variability recorded for pod/plant, followed by fruiting nodes/plant, germination 15 days after sowing, days to maturity, plant spread and harvest index. Greater variability was noted between crosses than between parents for all the traits except pods/plant and 100-seed wt. yield/plant, secondary branches per plant; 100 seed wt. and biological yield/plant appeared to be useful traits because high heritability and high genetic gain were recorded for theses characters.

Kumar *et al.* (1999) reported some 44 lines of lentil were evaluated for 12 quantitative traits over two environments at Hadwar during *rabi* 1995-1996. There was substantial genetic variability among genotypes for all the characters.

Singh *et al.* (1999) reported some 25 genotypes of lentil were studied for genetic variability and correlation of seed yield and other quantitative traits during *rabi* 1995-96. Days to 50% flowering, clusters/plant, pods/cluster seed/pod and harvest index showed significant positive correlation with seed yield.

and Hagwe

Chakraborty *et al.* (2000) reported studies on genetic variability and component analysis for quantitative traits were carried out in 48 genotypes of lentil. A high extent of genetic variation was observed for grain yield per plant 100-grain cut and number pods/plant.

and Sharma

Solanki *et al.*, (2001) reported healthy seeds i.e. 500, 1000 and 1500 of a *macrosperma* cv "Precoz selection"of lentil were treated with three rates (0.005,0.1 and 0.02) of N-trioso-N-ethyl-area, respectively on the basis of macromutations induced and inter and intra-family selection exercised in each treatment in M_2 generation was categorized into three different population *viz.* macromutatinal, selected and unselected. A wider range of variability for all the light polygenic traits than control in both positive and negative direction along with positive shift in character means in M_2 and M_3 generations was observed. Substantial amount of variability (CV) for all the traits was induced by different treatments in both M_2 and M_3 generation. Higher estimates of H_2 and GA, particularly in the selected and Macro mutational population in M_3 indicated tremendous scope for the improvement of seed yield and its component following selection.

Rathi *et al.* (2002) reported genetic variability related parameters were studied for eleven metric traits in 200 populations of lentil. There was considerable closeness between genotypic phenotypic coefficient of variation (GCV and PCV, respectively/indicating thereby low influence of environmental factors. GCV and PCV was low for days to flower, days to maturity and protein content contrary to this the traits number of primary branches/plant number of secondary branches/plant number of cluster/plant.

Hamdi *et al.* (2003) reported the genetic variability for earliness and seed yield characters were studied in 24 lentil genotypes. The

environments (Season and location) showed major effects on the performance of genotypes. High phenotypic variation was observed for number of pods and seeds/plant considering wide variability. Progress could be expected from selection for no. of seeds/plant and seed yield/plant. The early maturing genotypes Sinal, FLIP 87-212 and FLIP-92-54L could be recommended for planting in case the earliness in maturity is more important than seed yield. On the other hand, high – yielding genotypes FLLP89-71L, FLIP 95-68L, 89503, FLLP 92-48L and FLIP 95-50L could be recommended for planting in case high yield potential is more important than earliness in maturity.

2.3 Genetic divergence

Genetic divergence is the measure of genetic distance among the cultivars or germplasm lines. This divergence may be due to geographic barrier or any other reason, which may restrict the crossing, resulting in the formation of distinct groups. For creating variability crossing among the parental lines is the most potent and assured method. However selection of the divergent parents is most important aspect for this purpose, as greater the genetic divergence (distance) among the parents for the character (s) under consideration, better are the chances for release of variability and vice-versa. In view of the importance of this parameter, the breeders and geneticists must have information on genetic divergence among the breeding materials or germplasm lines under their custody. The Mahalanobi's D^2 analysis is the novel method for working out the genetic divergence and related statistics. In the following paragraphs the literature on genetic divergence in lentil is being reported.

Chahota *et al.* (1994) reported the nature and magnitude of genetic diversity was assed using. Mahalanobi's D^2Statistic and canonical

analysis for 15 trait in 40 *lens culinaris* genotypes of the small seeded type *(microsperma)* mostly of indigenous origin. Forty genotype were classified into six groups. Genotypes originating from a single country, such as India were assigned to different clusters. Based upon clustering pattern and performance, EC158884, EC158885, PL176 and PL 1268 were identified as promising parents for further breeding of *microsperma* lentil.

Rathi *et al.* (1998) reported twenty one lentil *(Lens culinaris)* varieties grown during the *rabi* season of 1992-93 and evaluated for 6 yield related traits. On the basis of D^2 analysis of the data obtained the varieties were grouped into 8 clusters number of primary branches contributed most to genetic divergence followed by yield/plant.

Solanki *et al.* (2000) reported seventy-two genotypes of lentil were grouped into eight and nine clusters under normal (E_1) and the late sown (E_2) conditions, respectively. The clustering pattern predicted that the genetic diversity is not necessarily parallel to the geographic diversity. The intercalate distance was maximum between clusters IV and VII followed by clusters II and VII in E_1. It was maximum between clusters III and IX, followed by clusters VIII and IX in E_2 suggesting that these groups of genotypes are highly divergent from each other. The genotypes in the above clusters of their respective environments revealed substantial differences in the means for important yield contributing characters. Therefore, considering the highest genetic distance, highest difference in cluster means for seed yield and maximum number of its attributes and performance of genotypes in both the environments, the crosses should be attempted between genotypes from cluster V and II in E_1 and clusters IV and IX in E_2.

Singh *et al.* (2001) reported the genetic divergence of 58 diverse strains of lentil was studied by multivariate analysis. A wide genetic distance was observed among the genotypes. As shown by the classification of the genotypes into 8 different clusters. Cluster VII contained 12 genotypes, while cluster VII contained 12 genotypes, while cluster III contained 2. the inter-cluster distance was lowest between clusters II and IV, and was highest between clusters III and VI.

Jeena *et al.*, (2001) reported Hierarchical cluster analysis was carried out involving 30 genotypes (28 wild accessions and 2 cultivars) of lentil, based on morphological and quality parameters. Three separate analysis were employed for qualitative (HCA1), quantitative (HCA2) and both qualitative and quantitative (HCA3) trait to work out the extent of genetic divergence. The results indicated wide genetic diversity as each analysis yielded formation of four, three and three clusters, respectively. Widest range of linkage i.e. Eclidian distances (Eds) were observed for cluster II followed by clusters I and III except in cases of HCA2 where cluster I showed highest range of Eds.

Jeena *et al.*, (2002) reported sixty-one wild lentil accessions representing all four species *Lens nigricans* (2), *L. odemnsis*. [*L culinaris subsp. odemensis*](16) *L.erviodes* (24) and *L. orientalis* [*L.culinaris subsp. orientalis*] (19) were evaluated in North-Indian climatic conditions at Pant nagar, Uttar Pradesh, India. The data were recorded on 20 quantitative trait and replication-wise means were subjected to Mahalonobi's D^2 analysis. All the accession was grouped into 4 clusters, in which cluster I included 58 accessions & while other clusters received only one accession each. It was also revealed that genetic diversity was not related to geographical diversity and species differences. Single seed

weight, leaf rachis length and percentage germination were the characters contributing most towards genetic divergence among the accessions.

Rakesh *et al.* (2002) reported the genetic diversity of 44 lentil cultivars in terms of 15 important characters was determined in a file experiment conducted in Hardwar, Haryana India during the *rabi* season of 1997 to identify suitable parents which can be used for the improvement of the crop. Genetic variation in the 44 cultivars for all the traits examined was observed. The cultivars were grouped in five clusters. Genetic distance was highest in cultivars grouped in cluster VIII and IX, in dictating greater divergence between cultivars belonging to these clusters.

Tejbir *et al.* (2004) reported D^2 analysis was carried out by in forty genotypes of lentil to study the genetic divergence during two successive years (1998-99) and (1999-2000) in Uttar-Pradesh, India. Analysis of variance (ANOVA) revealed significant differences among genotypes for all the characters except day to 50% flowering days to maturity and harvest index in both the years. The forty genotypes were grouped into six and seven clusters in the 1^{st} and 2^{nd} years, respectively. The relative composition of cluster differed in 2 years due to involvement of genotype × environment interactions. Clusters mean values indicated a wide range of variations for number of sec. branches/ plant in over two years on the basis of inter cluster distance and stability in the divergence; some genotypes were identified and proposed to be included in hybridization programme.

2.4 Correlation

Adequate knowledge about the nature and magnitude of association of characters is a pre-requisite for operating efficient selection programme. Exhaustive studies on interrelationship of characters among them and also between yield and its component traits have been carried

out in the past in many crops. However, such studies in lentil are limited, the same conducted very recently are being here under.

Zaman *et al.* (1989) reported yield correlation is derived from data on 8 yields components in 190 germplasm accession from Bangladesh. Correlation studies indicated that profusely branches plants with a high pod number had a high yield potential.

Ramgiry *et al.* (1989) reported yield correlations is derived from data on 6 and 5 yield components, respectively, in 21 varieties grown at Jabalpur India.

Rajput *et al.* (1989) reported correlation is derived from data on yield and 6 related traits in 22 accessions of diverse origin grown at one site.

Baidya (1988) reported phenotypic and genotypic correlations for 6 quantitative characters where studied in 96 lentil strains. Data revealed that the greater portion variance was due to environment. Seed wt/plt had the highest phenotypic, genotypic and environmental coefficient of variability.

Murari *et al.* (1988) reported Pusa 4 was or was not irrigated at IW; CPE (Irrigation Water: Cumulative Pan Evaporation) ratios of 0.2, 0.4, 0.6 or 0.8 and was or was not weeded. From simple correlation studies it was concluded that branches pod and seed number/plant, plant height and 1000-seed wt were positively correlated seed yield/plant.

Jain *et al.* (1991) reported multiple correlation and regression studies in 6 *Lens culinaris* varieties indicated that a combination of two or three variables such as height, branches/plant and pods/plant was the best combination for improving seed yield.

Esmail *et al.* (1994) reported seed yield was positively and significantly correlated with pod and seed number of plant ht. and number of branches/plant, and negatively with flowering duration.

Mohar *et al.* (1999) studies 25 genotypes of lentil were studied for correlation of seed yield and other quantitative traits like days to 50% flowering, clusters/plant, pods/cluster, seeds/pods, and harvest index.

Chauhan and Singh (2001) correlation analysis was conducted on 17 characters of 21 lentil genotypes. Seed yield was positively correlated with no. of secondary branches/plant, plant spread, number of fruiting lodes/plant and total biological yield/ fruit. In general these traits were also strongly correlated with each other.

Om *et al.* (2001) a study on correlation of yield and yield contributing characters was undertaken at two different locations using twenty eight advanced elite genotypes of lentil selected from *macrosprema* x *microsperma* crosses.

Rakesh *et al.* (2002) reported the analysis of inter-relationship among the 15 quantitative characters in 44 germplasm lines of lentil (*lens culinaris*). The correlation analysis indicated that general; the values of genotypic correlation were slightly higher than the corresponding phenotypic correlation values.

Sinha *et al.* (2002) reported correlation among various yield related traits was investigated in 21 genotypes of lentil (*Lens culinaris*) observation were recorded for plant height, (cm) branches/plant, pods/plant, seed/plant, pod length (pod width) seed diameter seed/plant and seed yield (g / plant).

Naji *et al.* (2003) reported a field experiment was carried out during (1996-97) at two locations on day loam soil in Jubeiha station Jordan, and on salty day in Mushagar research station, to evaluate a hundred exotic lentil cultivars for growth rate, yield and other agronomic traits. Seeds were sown by hand in 4-m length rows. Grain yield was positively correlated with biological yield, plant height yield/plant, seed/pod, pod, pods/plant and number of primary branches.

2.5 Path Coefficient analysis

In order to assess the direct and indirect effect of component trait on their dependent trait, the path coefficient analysis is of great value. The work done on this aspect in lentil is given below:

Nigem *et al.* (1990) reported the interrelation ships between yield and yield attributes as well as yield analysis were studied in eight intervarietal crosses and their parents. Positive and highly significant correlation coefficient was found between seed yield/plant on the one hand and number of pods as well as number of seeds/plant on the other hand.

Luthra *et al.* (1990) reported a study on correlation and path analysis was conducted on 56 lentil genotypes in the for 10 character-plant height, times to maturity, number of branches / plant, biological yield, number of pods/plant number of seeds/pods. number of seeds/plant, seed yield, 100-seed weight and harvest index. Path analysis revealed that biological yield is the main contributor towards seed yield other characters generally showed variation in their relative direct or indirect contributions.

Rasul *et al.* (1990) reported a study on path analysis with 70 selected lentil germplasm showed the grain yield was positively

correlated with days to flowering, days to maturity, plant height, pods per plant, seed per pod and harvest index.

2.6 Heritability

The knowledge of heritability is the most important parameter in designing selection schemes and improvement programme. It is an index of transmissibility of character(s) from parents to their off-springs. Lush (1940, 1943, 1949) has defined heritability both in broad and narrow senses. In broad sense, it is the ratio of genetic variance to the total variance or phenotypic variance $(V_G+V_D+V_I)/V_G+V_D+V_I+V_E)$ and in narrow sense, it is only a portion of genotypic variance which is due to additive $(V_G/V_G+V_D+V_I+V_E)$, where V_G,V_D,V_I and V_E are the variance due to additive, dominance, interaction (epitasis) and environmental effects, respectively. However, Robinson *et al.* (1949) defined heritability as the ratio of additive genetic variance and total variance expressed in percentage.

Smith (1952) described heritability as the ratio expressed in percentage of the variance component due to additive or fixable gene effects (σ^2G) to the sum of $\sigma^2G+\sigma^2D+\sigma^2E$, where σ^2G, σ^2D and σ^2E are the variances due to additive, dominance and environmental effects respectively. This parameter has also been defined as the square root of the correlation between the genotype and phenotype in an unselected population or the regression of the genotype on the phenotype (Robinson *et al.* 1949; Lerner, 1950; Smith, 1950).

There are several methods for estimating the heritability which can be broadly (classified as those based on (1) Parent-offspring regression; (2) variance components form an analysis of variance (3) approximation of non-heritable variance from genetically uniform populations to

estimate total genetic variance; (4) use of back-cross and F_2 populations as suggested by Warner (1952); (5) application of portioning or fractional method suggested first by Powers *et al.* (1950) and adopted later by Allard (1956); (6) the method of diallel crossing as suggested by Jinks (1954, 1955); Jinks and Hayman (1953) Allard (1956) and Dickinson and Jinks (1956) and (7) the constant parent regression method of Griffting (1950) which was originally defined by Hull (1948). Lerner (1950) categorized heritability estimates into low up to 10% medium between 10 to 20% and high above 20%.

Very limited studies have been done on this aspect in lentil. However, what so ever studies have been made on heritability in this crop is reviewed below-

Bhajan *et al.* (1987) reported a set of 68 crosses involving 17 strains, using partial diallel cross technique. Appreciable heterosis was observed for grain yield from 29.2 to 90.80% amongst crossed and from 1.04 to 159.31%.

Newaz *et al.* (1991) reported heritability and genetic advance were estimated as moderately high for yield but CVg and GA were relatively low for maturity.

Rasul *et al.* (1994) reported ten elite lines were evaluated for yield and yield-related trait in five cultural environments. A high heritability and high genotypic coefficient of variation with high expected genetic gain were found for days to flowering but not for days to maturity.

Chauhan *et al.* (1998) reported heritability were estimated for seed yield and 15 other characters in lentil *(Lens culinaris* Medik) experimental material included six parental lines are 15 F_1s. 100 seed

weight and biological yield per plant appeared to be useful trait because/recorded for these characters.

Rathi *et al.* (2002) reported heritability related parameters were studied for eleven metric traits in 200 populations of lentil. There were considerable closeness between genotypic and phenotypic coefficients of variation indicating thereby low influence of environmental factors.

Hamdi *et al.* (2003) reported heritability for earliness and seed yield characters were studied in 24 lentil genotypes. The environments showed major effect on the performance of genotypes. Considering wide heritability progress could be expected from selection for number of seeds/plant and seed yield/plant.

2.7 Genetic advance

The magnitude of genetic change that would result in next generation to selection is called genetic advance or genetic gain. This parameter depends upon:

1. The amount of genetic variability i.e. the magnitude of the differences in different individuals in the base population.
2. The magnitude of masking effect of the environmental and interaction components of variability of the genetic variability.
3. The intensity of selection (Comstock and Robinson1953).

The genetic gain in a character is a product of the heritability and the selection differential in units of standard deviation. Heritability value by itself does not have much significance. It is, therefore, necessary to utilize heritability estimates in conjunction with selection differential,

which would then indicate the expected genetic gain resulting from selection. In a directional selection, where the main objective of the breeder is to shift the mean of the population to a better standard, the expected gain would be a true indication of the effectiveness of the selection.

It is possible to determine expected genetic gain in a particular character as well as in an unselected character resulting from selection of either an individual character or a combination of characters in the form of a selection index.

Many breeders prefer to represent genetic advance in percentage of mean of a population, Burton and DeVane (1953) used the formula given below for estimating genetic advance in percentage of mean:

$$S = k \times \frac{Vg}{\sqrt{V p \times \bar{X}}} \times 100$$

or

$$\text{Genetic advance} = \frac{(G.A)}{\text{Grand mean } (\bar{X})} \times 100$$

Where,

S = Genetic advance in percentage of mean.

k = Selection differential at a particular selection intensity i.e. 2.06 at 5% selection intensity.

Vg = Genetic variance.

Vp = Phenotypic variance.

$\bar{X}$ = Mean value of the character x

Bhajan *et al.* (1987) reported a set of 68 crosses involving 17 strains using partial diallel cross technique. The best yielding hybrids were late flowering, but earlier in maturity than their respective better yielding parents.

Newaz *et al.* (1991) reported expected genetic advance were estimated as moderately high for yield but CVg and GA were relatively low for maturity.

Rasul *et al.* (1994) reported ten elite lines were evaluated for yield and yield related traits in five cultural environments. The genotypic coefficient of variation and expected genetic gain were moderate for number of pods/plant but low for harvest index and number of seeds/pod and nodules/plant. The phenotypic coefficient of variation for yield was quite high but showed low heritability.

Chauhan *et al.* (1998) reported expected genetic advance were estimated for seed yield and 15 other characters in lentil yield/plant, secondary branches/plant, 100-seed weight and biological yield/plant appeared to be useful traits because high heritability and high genetic gain were recorded for these character.

Rathi *et al.* (2002) reported estimated genetic advance for eleven metric traits in 200 population of lentil. 1000-grain weight and yield/plant showed the success in selection estimated in the form of genetic advance over mean ranged from 12.2% to 35.1% however estimated 8.4% in F_1 and 4.6% in F_2.

Hamdi *et al.* (2003) reported genetic advance for earliness and seed yield character were studied in 24 lentil genotypes. Considering wide heritability and genetic advance, progress could be expected from selection for number of seeds/plant and seed yield per plant.

2.8 Molecular Markers

The potential usefulness of genetic markers as a screening tool in plant breeding was well recognized by Sax (1923). The application of morphological markers in developing linkage map was largely hindered due to lack of ability to estimate genetic distances, inadequate knowledge of genetic control and a time consuming. However, the development of biochemical and molecular markers (isozyme, and more recently, DNA markers) promise to overcome the limitation of morphological markers they have several advantages over morphological marker because of:

a. Abundance through the whole genome.

b. DNA markers usually not affected by environment.

c. Basis of inheritance can be understood.

d. No influence of penetrance and expressivity.

e. Tissue and stage-independent expression.

f. Rapid screening.

g. Applicability across the species.

In the recent past isozymes have been used successfully in certain areas of plant breeding and genetics as almost environmentally natural genetic marker (Tanksley and Orton, 1983).Unfortunately, their low level of polymorphism and issue-specific expression have limited their practical utility (Tanksley,1993; Tatineni *et al.*, 1996).

On the other hand, polymorphism in nucleotide sequence is obviously much frequent. This polymorphism is revealed by different molecular maker techniques, such as restriction fragment length polymorphism (RFLP), amplified fragment Length polymorphism

(AFLP). randomly amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequence (CAPS) and sequence characterized amplified region (SCAR).

The development of polymerase chain reaction (PCR) by Mullis (1985) has revolutionized many standard molecular biological techniques on such variation generates a specific class of molecular marker which was termed as randomly amplified polymorphic DNAC (RAPD) (Williams *et al.*, 1990; Weish and McClelland, 1990).

RAPD analysis has been demonstrated to be an efficient marker detection system, particularly for disease resistance gene(s). The tightly linked RAPD marker, if available, are very useful and efficient in marker assisted selection (Michel more *et al.*, 1991). Although the RAPD markers are reported to be less reliable as compared to RFLPs (Weeden *et al.*, 1992) but they have reproducible of RAPD markers based on the result of result of several reproducible provide that all the details of reaction conditions are standardized in minute details and strictly adhered to. Based on these studies, they reported 75 percent reproducibility of RAPD markers.

Advances in molecular biology have introduced an alternative for genotype characterization and identification. The biochemical and molecular markers have been recently used in genetic characterization of the germplasm in a number of crops. These markers are useful tools in generating important information that may enhance the use of germplasm in the crop improvement programme. In lentil (*Lens culinaris* Medik) more than 100 markers have been listed with morphological and physiological characteristics. But these markers are not reliable as they are liable to be influenced by the environmental factors. Therefore, the molecular markers are better alternatives for characterization and identification of

germplasm/cultivars. In the following lines the literature recently available on all the facts of molecular markers in lentil has been reviewed.

The potential use of genetic markers to establish linkage maps has increased dramatically over the last two decades. Relatively well developed linkage maps that include loci encoding various isozymes, molecular markers, qualtitative and quantitative trait loci are available in several crops such as maize (Coe *et al.,* 1990) wheat (Hart and Hale, 1990) Pea (Weeden and Wolko 1990) and lentil (Tahir *et al.,* 1993).

An important use of these maps would be to develop marker assisted selection in plant breeding. Several isozymes and molecular marker (RAPD, RFLP,SSR) closely linked to the gene of interest have been mapped in their respective linkage groups. For example, an alcohol dehydrogenase locust (Adn-1) was linked to a gene for resistance to pea enation mosaic virus (*En*) in pea (Weeden and Providenti, 1987) linkage between RFLP locus and disease resistance gene has been reported in tomato (Sarafatti *et al.*, 1989).

Torres *et al.*, (1993) used segregating allozyme and polymorphism to construct a preliminary linkage map for faba bean. Two F_2 populations were analyzed, the most informative of which was segregating for 66 markers. Eleven independently assorting linkage groups were identified by them. One of the groups contained 45s ribosomal array and could be assigned to large met centric chromosome 1, on which the molecular marker nucleolar organizer region (NOR) was located. This linkage group also contained two isozyme loci, *Est* (esterase) and *Tpip* (triosephosphate isomerase), suggesting that it may share some homology with chromosome 4 of *Pisum sativum* on which three similar markers are

syntenic. They also discussed additional maps and the extent of the coverage of the total marker genome.

Muehlbauer *et al.* (1993) reported the potential of wild species of chickpea lentil, pea and *vicia faba* to provide the genes heeded for resistance to stresses. The barriers to transferring these gene and methods for overcoming obstacles to gene transfer, as well as the transfer of specific segment of a genome facilitated by molecular markers, are discussed. Information is arranged under the following headings: origins and wild relatives of cool season food legumes; germplasm collections of wild species; gene pools of cool season food legumes, Interspecific hybridization in cool season food legumes; introgression based on molecular markers and pre-breeding.

Tahir *et al.* (1993) reported morphological and molecular markers have been reviewed for the purpose of establishing a preliminary linkage map for lentil (*Lens culinaris*) the map includes 7 morphological, 25 isozymes, 38RFLP and six other loci. The map is arranged in order to take into account the reported homology of linkage groups among the *vicieae* species. There are currently ten recognized linkage groups, which should currently ten recognized linkage groups, which should eventually be reduced to seven as mapping continues. An alphabetical list of polymorphic loci is presented with respect to linkage group, description and references.

Weeden *et al.* (1994) reported an understanding of the genetic basis of commercially important characters is critical when a breeder is attempting to incorporate such characters into breeding material. Some particularly interesting characters of genes have been identified in cool season food legumes, and in pea many of these have been tagged with

molecular markers such as allozyme or DNA polymerase. This process of mapping and tagging genes has been greatly accelerated by recent developments in molecular biology. It appears that markers will soon be available for many genes in lentil, faba bean and chickpea and that genetic knowledge developed in one crop will have significant applications in the other cool season food legumes.

Ahmad and Mc Neil (1996) reported the phylogenetic relationship among different subspecies of the genus *Lens* using morphological (quantitative and qualitative character) biochemical (SDS-PAGE) and molecular RAPD markers. There was both a substantial level of agreement and disagreement between the reports based on different analytical procedures and different germplasm. *Lens culinaris* ssp. *Orientalis* appeared as the wild progenitor of the cultivated lentil crop evolution was suggested. It was also suggested that these techniques could be used in combination for taxonomic analysis of the genus *Lens*.

Eujayl *et al.*, (1997) reported that to maximize the extent of polymorphism within a mapping population wide crosses are often made to know the segregation distortion. They made the cross between wild lentil (*Lens culinaris* subsp. *Orientalis*) and cultivated lentil (*Lens culinaris* Medik) and RAPD marker were used for genetic mapping in F_2 population. Eighty three percent RAPD markers shown segregation distortion, which was also observed in isozyme and morphological loci. seventy eight segregating loci were analyzed for linkage at a LOD score >3.0, resulted in 28 RAPD one RFLP, one morphological and three oligonucleotide markers, which were assigned to a linkage, group spanning 206 cm of lentil genome. It's indicates that RAPD markers were valuable for genetic mapping and evaluation of segregation distortion in lentil.

Ford *et al.* (1997) reported random amplified polymorphic DNA (RAPD) analysis, the relationship between 16 accessions and cultivars of lentil (*Lens culinaris*) were examined in the Australian lentil breeding programme. All lines exhibited polymorphism with a maximum dissimilarity value of 0.36. This indicated a limited degree of genetic variation. Polymerase Chain Reaction (PCR) with primers based on the flanking regions of the 5S rRNA gene from *pisum sativum* amplified the non-translated spacer (NTS) region from within the 5S rRNA gene of *Lens*. Three distinct amplification banding patterns differentiated between restricted genomic DNA of *Lens* spp. L-*culinaris* subsp. *culinaris* and *L-culinaris* subsp. *orientalis* [*L.orientalis*] shared similar markers of two distinctly different NTS sizes. *L. nigricans* and *L. odomensis* shared the same amplification pattern of a single sized NTS region. However, Lervoides contained two separate sizes of NTS, distinct from other *Lens* species. In an effort to widen the genetic base of cultivated lentil, these species-specific molecular markers may be used to follow potential introgression between species.

Simon and Muehlbauer (1997) developed an integrated genetic linkage map of chickpea, which consist of a morphological, 27 isoenzymes 10RFLP, and 45RAPD markers covering 550 cM. The linkage map, so constructed, has 10 linkage groups representing the eight chromosomes of chickpea. Several regions of genome were slighting skewed from the expected Mendelian ratio of alleles. Lentil genomic DNA (RFLP probes) hybridized poorly to chickpea DNA, indicating considerable divergence of these genomes at the molecular level.

Tiwari *et al.* (1998) reported bulk segregant analysis screened 416 primers, of which amplicons of three operon primers; OPO18, OPE16, and OPL6, were found to be linked to er1. OPO18 1200 was linked in

coupling (trans to er1) phase and OPE16 1600 (4ft2cm.) and OPL16 900(2ft cm) were linked in repulsion (cis toer-1) phase.

Bhat *et al.* (1999) analyzed 58 accession of sesame (*Sesamum indicum L.*) using Random Amplified Polymorphic DNA (RAPD) technique. They statistically analysed the results from PCR amplifications with the selected 24 random decamer primers. The value of Jaccard's similarity coefficient ranged from 0.19 to 0.89. The result indicated the presence of high level of genetic diversity. However, the extent of genetic diversity was greater in the collections from Rajasthan and North-eastern and the remaining highly diverse accession were placed outside these close knit clusters. The bootstrap estimates obtained by Wagner parsimony analysis were significant for 7out of 49 nodes of in the majority rule consensus tree. The results of the individual clusters were considered. The principle component analysis indicated that the first two components accounted for only 21% of the total variations and in order to explain >75% of variation 18 component were required. The high level of genetic diversity prevalent among the Indian collections was reported probably the indicative of the nativity of this crop.

Croft *et al.* (1999) used eight *Lathyrus sativus* accessions from a variety of geographic origins to study intraspecific genetic diversity using RAPD analysis. Fourteen decamer primers produced 64 amplification products, 50% of which were polymorphic between the samples the dendogram showed that most of the plants were clustered into accessions of common geographical area.

The average genetic similarity coefficient within accession was 0.12 and between accession was 0.20, indicating a low level of intraspecific genetic variation. Interspecific genetic diversity and phylogenetic relationship

of eight *Lathyrus* species, including *L. sativus* were examined using 14 decamer primers, which produced 28.3 amplification products. In the dendrogram, the *Lathyrus* species clustered into three distinct groups, which are correlated with the sections *Lathyrus clymenum* and *Lineoricarpus*. This supports traditional taxonomic classifications of the genus *Lathyrus* that are based on morphological traits.

Rubeena *et al.*, (2003) reported first intraspelific linkage map of the lentil genome was constructed with 114 molecular markers (100 RAPD, 11 SSR and 3 RGA) using an F_2 population developed from a cross between lentil cultivars ILL5588 and ELL 7537 which differed in resistance for ascochyta blight. Linkage analysis at a LOD scores of 4.0 and maximum recombination fraction of 0.25 revealed nine linkage groups comprising between 6 and 18 markers each. The infraspecific map spanned a total length of 784.1cm the markers were clustered in the middle or near the middle of the linkage groups, suggesting the location of centromeres of 114 mapped markers, 16 (14.08% were distorted, usually at the end or middle of the linkage groups. The utility of ISSR and RGA markers for mapping in lentil was explored, and the primer with a (AC) repeat motif was found to be useful.

MATERIALS AND METHODS

The materials used and the methods followed for conducting the proposed experiments under the present investigation **"Charactacterization of lentil germplasm through morphological and metric traits and molecular markers"** are detailed below:

3.1 Materials

The test material for the present study was a set of 100 germplasm lines and varieties belonging to the genus lentil. However, the accessions belonging to former species were larger in number than those of the later. These accessions were selected from the Lentil Germplasm being maintained at the Division of Genetics, Indian Agricultural Research Institute, New Delhi on the basis of variability observed in their morphological and qualitative character (Table 3.1). The symbol L1 to L100 have been assigned to these accessions (Table 3.2), which was used throughout the text of the thesis, for facilitating the presentation and description of results. However, wherever the original number was required, it has been quoted accordingly.

3.2 Raising of the material

The selected 100 germplasm accessions and varieties including three checks viz. Precoz, Sehore74-3, and L 4076 were grown in the augmented randomized complete block design at the Research Farm of the Indian Agricultural Research Institute, New Delhi during *rabi* season of 2003-2004. Each accession was represented by 3 m long row. The spacing between two consecutively sown accessions (row to row) was kept at 50 cm. The spacing between the plants within the row was maintained at 15 cm. The three checks were repeated, after every 20th entry.

Table 3.1 Details of the parental strains lentil used in the present investigation.

S.No.	Accession	Pedegree/line	Source	Origin
1.	Sehore 74-3	Local selection from MP	Sehore	JNKUV, Jabalpur
2.	LH90-57	Sehore 74-3 × K-75	HAU	HAU, Hisar
3.	L 830	Selection from germplasm	IARI	IARI, New Delhi
4.	JLS 1	Unknown	JNKV, Jabalpur	JNKV, Jabalpur
5.	LC93-5-3-3-1	Precoz X Sehore 74-3	IARI	IARI
6.	L 4149	RAU101 × PL 639	IARI	IARI
7.	E 153	Selection from germplasm	GBPPUA&T	Pantnagar
8.	L 1304	Germplasm	IARI	IARI
9.	L 4147	L3875 × P4 × PKVL-1	IARI	IARI
10.	L 4378	Germplasm	IARI	IARI
11.	L 4384	Germplasm	IARI	IARI
12.	L 435	Germplasm	ICARDA	ICARDA, Syria
13.	SKL 259	Germplasm	IARI	IARI
14.	10-2-B-2	LC74-1-5-1 × LC68-17-3-5	IARI	IARI
15.	L 3685	ILL1465	ICARDA	Iran
16.	L 4387	Germplasm	IARI	IARI
17.	LC74-1-5-1	Lens3877 × pusa4 × PKVL-1	IARI	IARI
18.	PUSA-4	Selection from local germplasm	IARI	IARI
19.	PL 639	L9-12 × T8	Pantnagar	Pantnagar
20.	L 4603	Precoz × PL77-12	IARI	IARI
21.	L 4602	Precoz × Shehore74-3	IARI	IARI
22.	Fasciated Mutant	Mutant of L830	Meerut University	Meerut University

Contd.

23.	Dwarf Mutant	Unknown	Meerut University	Meerut University
24.	8-1	LS106 × L263	IARI	IARI
25.	Globe Meerut	Mutant of L830	Meerut University	Meerut University
26.	10-3Y-26 Normal	LC74-1-5-1 × LC68-17-3-5	IARI	IARI
27.	19-B-5 globe	L830 globe × LC 74-1-5-1	Meert university	Meerut University
28.	19-B-5 Normal	L830 globe × LC 74-1-5-1	IARI	IARI
29.	L 4605	L4084 × L3991	IARI	IARI
30.	L 6183	Precoz × PL-406	IARI	IARI
31.	PL 406	Selection from P4 95	IARI	GBPUAT, Pantnagar
32.	LC68-17-4-2	L4084 × L3991	IARI	IARI
33.	E C383090	Selection from germplasm	ICARDA	ICARDA
34.	E C383087	Selection from germplasm	ICARDA	ICARDA
35.	P 22211	Selection from germplasm	ICARDA	Unknown
36.	P 33159	Selection from germplasm	ICARDA	Unknown
37.	14-1-y-50	LC68-17-3-5 × L4602	IARI	IARI
38.	L 4076	PL234 × PL639	IARI	IARI
39.	Precoz	ILL-4605	ICARDA	Argentina
40.	MC-6	Selection from germplasm	ICARDA	ICARDA
41.	MC-1	Selection from germplasm	ICARDA	ICARDA

Contd.

42.	25-26	Lens 4602 × Lens 6163	IARI	IARI
43.	L68-17-3-5	Precoz × L3931	IARI	IARI
44.	8-3	LS 106 × L263	IARI	IARI
45.	ICARDA PROMISING	Selection from ICARADA germplasm	ICARDA	ICARDA
46.	EC383084	Selection from germplasm	ICARDA	Unknown
47.	P22127	Selection from germplasm	ICARDA	Unknown
48.	RL-1	Selection from germplasm	IARI	IARI
49.	P22107	Selection from ICABA germplasm	ICARDA	ICARDA
50.	L-5258	Precoz × L4636	IARI	IARI
51.	PKVL-1	Selection from germplasm	IARI	PKV, Akola
52.	P 22115	Selection from germplasm	ICARDA	Unknown
53.	10-3Y-26 globe	LC74-1-5-1 × Globe mutant	IARI	IARI
54.	FLIP-96-51	ICARDA	ICARDA	Unknown
55.	PL 5	Selection from germplasm	Pantnagar	Pantnagar
56.	DPL 21	Unknown	IIPR	Kanpur
57.	DPL 15	PL406 × L4076	IIPR	Kanpur
58.	DPL 62	JLS 1 × LG171	IIPR	Kanpur
59.	IPL 81	K75 × PL639	IIPR	Kanpur
60.	L 5120	Precoz × PKVLI	IARI	IARI
61.	L 5214	ILL 6037	ICARDA	ICARDA
62.	L 7481	Selection from germplasm	ICARDA	ICARDA

Contd.

63.	L 3214A	ILL 6037	ICARDA	ICARDA
64.	L 1195-1770	Selection from germplasm	IARI	IARI
65.	L 5214B	ILL 6037	ICARDA	Unknown
66.	L 97-454-1	Selection from germplasm	IARI	IARI
67.	L 5115	ILL 6037 × JLS-1	ICARDA	Syria
68.	L 6171	L4076 × LH84-8	IARI	IARI
69.	L 4636	Precoz × L3991	IARI	IARI
70.	L 5227	L4603 × PKVL-1	IARI	IARI
71.	L 5086	LC6317-3-5 × L4602	IARI	IARI
72.	P 3101	Selection from germplasm	ICARDA	Syria
73.	P 19132	Selection from germplasm	ICARDA	Syria
74.	L 7476	Selection form germplasm	ICARDA	Turkey
75.	L 7417	Selection from germplasm	ICARDA	Syria
76.	L 7462	Selection from germplasm	ICARDA	Syria ICARDA
77.	L 5228	ILL8006 × ILL1961	ICARDA	Syria
78.	P 3136	Selection from germplasm	ICARDA	Syria
79.	L 5084	L4147 × JLS- 1	IARI	India
80.	6/7	Unknown	IARI	Unknown
81.	L 4595	L4147 × PL406	IARI	India
82.	L 7474	Selection from germplasm	ICARDA	Syria
83.	L 4592	L830 × PKVL- 1	IARI	IARI, New Delhi
84.	6-59	Unknown	IARI	IARI

Contd.

85.	L 7461	Selection from germpasm	ICARDA	Syria
86.	L 7463	Selection from germplasm	ICARDA	Syria
87.	L 7473	Selection from germplasm	ICARDA	Syria
88.	L 7459	Selection from germplasm	ICARDA	Syria
89.	P 3107	Selection from germplasm	ICARDA	ICARDA
90.	K 75	Local selection from Bundelkhand	Bundelkhand	Unknown
91.	P 2180	Selection from germplasm	ICARDA	Unknown
92.	P 2215	Selection from germplasm	ICARDA	Unknown
93.	L 7354	MC-6 × Sehore 74-3	IARI	IARI
94.	L 4614	L4076 × L830	IARI	IARI
95.	L 6173	RAU101 × PL639	IARI	IARI
96.	P 19235	Selection from germplasm	ICARDA	Syria
97.	P 2228	Selection from germplasm	ICARDA	Syria
98.	P 3111	Selection from germplasm	ICARDA	Syria
99.	L 7358	MC-6 × Sehore74-3	IARI	IARI
100.	L 7449	Selection from germplasm	ICARDA	ICARDA

Table 3.2 **The germplasm lines and varieties of lentil and symbol used in the experiments**

S.No.	Accession	Symbol used	S.No.	Accession	Symbol used
1	Sehore74-3	L1	29	L 4605	L 29
2	LH90-57	L 2	30	L 6183	L 30
3	L 830	L 3	31	PL 406	L 31
4	JLS-1	L 4	32	LC68-17-4-0	L 32
5	LC93-5-3-3-1	L 5	33	EC 383090	L 33
6	L 4149	L 6	34	EC 383087	L 34
7	E 153	L 7	35	P 22211	L 35
8	L 1304	L 8	36	P 33159	L 36
9	L 4147	L 9	37	14-1-Y-50	L 37
10	L 4378	L 10	38	L 4076	L 38
11	L 4384	L 11	39	Precoz	L 39
12	L 435	L 12	40	MC 6	L 40
13	SKL-259	L 13	41	MC 1	L 41
14	10-2-B-2	L 14	42	25-26	L 42
15	L 3685	L 15	43	LC 68-17-3-5	L 43
16	L 4387	L 16	44	8-3	L 44
17	LC74-1-5-1	L 17	45	ICARDA PR0MISING.	L 45
18	Pusa-4	L 18	46	EC 383084	L 46
19	PL 639	L 19	47	P 22127	L 47
20	L 4603	L 20	48	RL 1	L 48
21	L 4602	L 21	49	P 22107	L 49
22	Fasciated mutant	L 22	50	L 5258	L 50
23	Dwarf mutant	L 23	51	PKVL 1	L 51
24	8-1	L 24	52	P 22115	L 52
25	Globe meerut	L 25	53	10-3Y-26 globe	L 53
26	10-3Y-26 normal	L 26	54	FLIP-96-51	L 54
27	19-B-5 globe	L 27	55	PL 5	L 55
28	19-B-5 normal	L 28	56	DPL 21	L 56

Contd.

57	DPL 15	L 57	79	L 5084	L 79
58	DPL 62	L 58	80	6/7	L 80
59	IPL 81	L 59	81	Rajendra II	L 81
60	L 5120	L 60	82	L 7474	L 82
61	L 5214	L 61	83	L 4592	L 83
62	L 7481	L 62	84	6-59	L 84
63	L 5214 A	L 63	85	L 7461	L 85
64	L1195-1770	L 64	86	L 7463	L 86
65	L 5214 B	L 65	87	L 7473	L 87
66	L97/454-1	L 66	88	L 7459	L 88
67	L 5115	L 67	89	P 3107	L 89
68	L 6171	L 68	90	K 75	L 90
69	L 4636	L 69	91	P 2180	L91
70	L 5227	L 70	92	P 2215	L92
71	L 5086	L 71	93	L 7354	L93
72	P 3101	L 72	94	L 4614	L94
73	P 19132	L 73	95	L 6173	L95
74	L 7476	L 74	96	P 19235	L96
75	L 7477	L 75	97	P 2228	L97
76	L 7462	L 76	98	P 3111	L98
77	L 5228	L 77	99	L 7358	L99
78	L 3136	L 78	100	L 7479	L100

All recommended growing practices for raising a healthy and normal crop of lentil, such as dose of fertilizers and its method of application, hoeing and weeding, irrigation, and plant protection measures, etc. were adopted.

3.3 Recording of the observations

3.3.1 Morphological characters

The crop being autogamous and has been maintained continuously for the last several year, each accession was phenotypically uniform and genetically pure and hence no variation in morphological features within the genotype under study was observed. Therefore, the observations on the following morphological characters were recorded not on individual plant basis, but on the basis of the line as a whole.

1. Growth habit

Two groups of growth habit viz. erect and prostrate were considered. All germplasm lines not showing erect habit of growth irrespective of extent of spreading nature were grouped into prostrate class.

Prostrate growth habit plant have profuse brancheses and the main stem are very short. Whereas, the erect growth type plant have long main stem with minimum branches. At early stage of growth it can be easily distinguished phenotypically between prostrate and erect growth habit. Data were recorded at 45-55 days after sowing. But at the later stages when temperature raise and lentil plants are in fast growing stage, the lower branches of prostrate plants also grow upwards and at this stage difficulties may arise in classification of such plants either prostrate or intermediate types. Therefore, careful observation is needed in appropriate time of growth stage. There were several mutants like globe, stunted, bushy dwarf etc. for growth habit.

2. Plant pubescence

The presence or absence of pubescence (hairy structure) on different plant parts was recorded at 35-50 days after germination. It was observed that the plant, which was having pubescent for leaf, was also pubescent for stem and pod. Some genotypes were highly pubescent which could be recognized very easily. However, some difficulty may be encountered in case of less pubescent genotypes. Non-pubescent or glabrous type of plants have almost clean and smooth surface.

3. Stem pigmentation

Presence or absence of anthocyanin pigmentation on stem was recorded during early stages of crop growth (35-50 days after sowing) because at later stages the pigment may disappear due to environmental influence and may pose difficulty in recording observations.

4. Leaf pigmentation

The anthocyanin pigmentation of leaf is a stage and environmental dependent trait. There is no difference between pigmented and non-pigmented genotypes at very early stage of seedlings (3-4 weeks old). The leaves of pigment-potent plants start turning brown and remain pigmented till about flowering time after which the brown pigment disintegrates and the plants look like a non-pigmented type.

Since expression of leaf pigmentation is stage dependent, each genotypes was observed for at least two times during its lifetime beginning from 40 days after sowing at 15 days intervals. Within this period all the pigment-potent plants manifested leaf colouration and subsequent observation for this trait was not necessary.

5. Tendril formation

The data were recorded at the crop maturity stage on the basis of presence or absence of tendril at the leaf apex. All the branches of the plants were observed carefully because sometimes-rudimentary tendril could also be formed on the tendril-potent plants.

6. Leaf shape

This character was determined on the basis of shape or form of leaf apex. More flat or more wide leaf corner was considered the oval shape and more sharp or less flatten form of leaf was treated as acute shape of leaf observation was recorded at 35-45 days after germination.

7. Leaf size

This character was recorded on the basis of morphological structure of the leaf. The leaf size was decided based on the length and width of the leaflets. The genotypes parents with leaflets length of 20-22 mm and width 7-10 mm were designated as broad leaflets, whereas genotypes having leaflets length of 10-11 mm and width 3-4 mm were categorized under narrow leaflets. Data was recorded at the vegetative phase of the plant i.e. within 35-45 days after germination.

8. Leaf colour

This character was determined on the basis of greenness of leaf at maturity. Deep greenish leaf was considered as dark green colour while light green or light yellowish leaf was treated as light green colour (Mishra *et al.*, 2001).

9. Stipule size

The stipules having leaflet like structure were taken as long stipule and those modified into vestigial from, irrespective of extent of reduction in

size, were classified as short stipules. Accordingly, the germplasm accessions were put into two categories (i.e. short stipules and long stipule).

10. Peduncle length

The two categories of peduncle length viz, short and long were made based on visual length of peduncle. The observations were recorded at full podding stage.

11. Flower colour

The character was determined on the basis of colour of the flowers. Generally white, pink, purple, blue colours of flowers were available in lentil genotypes. The data were recorded at the time of flowering stage.

12. Testa colour

Observation for testa or seed coat colour was recorded after harvesting the seeds at maturity the expression of testa colour was likely to be affected by the colour of cotyledon of specific seed. For example, colourless testa or testa in combination with yellow, green and orange cotyledons seems to be yellowish, light green and brown, respectively. According to Erskine and Witcombe (1984), the background colour of seed cat may be black, brown, gray pink or green, tan, buff, yellow.

13. Testa pattern

Spotting pattern on seed coat was recorded after harvesting the seeds. The observations were recorded either for presence or absence of pattern on the seed coat as well as for the nature of pattern. Seeds having small and uniform spots were called as mottled, while the seeds with large and irregular spots were named as speckled. When both the types of spotting were present on the same seed coat, it was called combined. However, only

mottled and non-mottled seed coat pattern has been considered in the present investigation.

14. Seed shape

The three categories of seed shape viz. flat, spherical, oval, were recorded based on the actual shape of seed after harvest.

15. Cotyledon colour

After harvesting the seeds the observations on cotyledon colour were recorded. Screening of cotyledon colour is possible without removing the testa of a particular seed by a seed-screening device. The seeds were exposed to light of the device and light pass through the papery testa which represent the colour of the cotyledon. Although, it was an easy technique but the resolution to identify the perfect colour of cotyledon was very high. Difficulty may arise when seeds with dark black or densely mottled testa pattern are used. Five different colours of cotyledon viz. orange, yellow, brown, dark green and light green were observed in the present study.

3.3.2 Data on quantitative characters

For recording observations on quantitative characters, five plants were randomly selected in each accession. On each of these plants the observations were recorded on the following characters in the manner as described in each case, individually:

1. Days to flowering

The date on which flowering initiated was noted in each accession and the number of days taken from sowing to appearance of flower were calculated as days to flowering.

2. Days to maturity

The date on which all the pods matured physiologically and the plant turned pale yellow was noted in each germplasm line and variety. The number of days taken from sowing to maturity was worked out accordingly.

3. Plant height (cm)

The plant height was measured with the help of scale (cm) form soil surface to apex of each tagged plant and the average was worked out.

4. Number of flowers per peduncle

Numbers of flowers per peduncle were recorded on individual plant based on maximum number of flowers found per peduncle in a particular plant. For example, even if one peduncle in a plant had five flowers that plant was classified as five flowered plant. Assuming that the particular plant has the ability to produce five flowers on one peduncle.

5. Number of pods per plant

The number of pods in each plant was counted and the average was worked out by dividing number of sampled plants.

6. Number of seeds per pod

The number of seeds in the first pod, which was measured for length, was counted after threshing.

7. 100-seed weight (g)

The grain produce of the five plants of each plant was mixed and the weight of randomly selected 100 grain was recorded in grains.

8. Grain yield/plant (g)

The weight of all the grains harvested in each plant was recorded in g as grain yield per plant.

9. Biomass score (g)

The total plant weight including straw and grain was considered as the total biomass score per plant. This was recorded in grams.

10. Harvest index (%)

The harvest index (%) is the ratio of grain yield to the total biomass score and was computed by the formula as follows

$$H.I = \frac{\text{Grain yield per plant}}{\text{Total biomass score}} \times 100$$

3.3.3. Molecular markers

1. Plant material

Out of 100 germplasm lines grown during 2003-04, 60 lines as given in (Table 3.3) were selected for RAPD analysis. The selection of these 60 accessions and varieties was based on the criteria that as far as possible these lines should represent total variability in germplasm. Again, these lines were raised in augmented randomized complete block design with, Sehore74-3, Precoz, L 4076 as checks during *rabi* 2004-05 at Research Farm of the Indian Agricultural Research Institute, New Delhi. The lay out plan, agronomic practices and plant protection measures followed were the same as were adopted during 2003-04.

Table 3.3 Germplasm lines and varieties of lentil used for RAPD analysis

S.No.	Accession/Variety	Symbol used	S.No.	Accession/Variety	Symbol used
1	Sehore74-3	L1	31	PL 406	L 31
2	LH 90-57	L 2	32	LC 68-17-4-0	L 32
3	L 830	L 3	33	EC 383090	L 33
4	JLS 1	L 4	34	EC 383087	L 34
5	LC 93-5-3-3-1	L 5	35	P 22211	L 35
6	L 4149	L 6	36	P 33159	L 36
7	E 153	L 7	37	14-1-Y-50	L 37
8	L 1304	L 8	38	L 4076	L 38
9	L 4147	L 9	39	Precoz	L 39
10	L 4378	L 10	40	MC 6	L 40
11	L 4384	L 11	41	MC 1	L 41
12	L 435	L 12	42	25-26	L 42
13	SKL 259	L 13	43	LC 68-17-3-5	L 43
14	10-2-B-2	L 14	44	8-3	L 44
15	L 3685	L 15	45	ICARDA PROMISING	L 45
16	L 4387	L 16	46	EC 383084	L 46
17	LC 74-1-5-1	L 17	47	P 22127	L 47
18	Pusa-4	L 18	48	RL 1	L 48
19	PL 639	L 19	49	P 22107	L 49
20	L 4603	L 20	50	L 5258	L50
21	L 4602	L 21	51	PKVL 1	L 51
22	Fasciated mutant	L 22	52	P 22115	L 52
23	Dwarf mutant	L 23	53	10-3Y-26 globe	L 53
24	8-1	L 24	54	FLIP 96-51	L 54
25	Globe mutant	L 25	55	PL 5	L 55
26	10-3Y-26 normal	L 26	56	DPL 21	L 56
27	19-B-5 globe	L 27	57	DPL 15	L 57
28	19-B-5 normal	L 28	58	DPL 62	L 58
29	L 4605	L 29	59	IPL 81	L 59
30	L 6183	L 30	60	L 5120	L 60

2. Plant DNA extraction

2.1 Reagents for plant DNA isolation

(i) 1M Tris-HCl (pH 8.0):121.1g Tris-HCl salt was dissolved in 800 ml of double distilled water and pH adjusted to 8.0 using 0.1N NaOH. The volume was made up to 1 lit and the content was autoclaved.

(ii) 0.5M EDTA (pH 8.0): 186.1g sodium salt of EDTA was dissolved in 800 ml of double distilled water and pH adjusted to 8.0 using NaOH pellets.

(iii) 4M NaCl: 233g sodium chloride was dissolved in double distilled water and volume was made up to 1 lit. and autoclaved.

(iv) 10%: 50 g cTAB was dissolved in sterile double distilled water and volume was made up to 500 ml.

2.2 Composition of DNA extraction buffer

(i)	Tris-HCl (pH 8.0)	100 mM
(ii)	Na-EDTA salt (pH 8.0)	20 mM
(iii)	NaCl	1.4M
(iv)	cTAB	2%(w/v)
(v)	B-mercaptoethanol	0.2%

2.3 Preparation of DNA extraction buffer

5.0 ml of 1M Tris-HCl, 2.0 ml of 0.5M EDTA, 17.5 ml of 4M NaCl and 10.0 ml of 10% cTAB were mixed together to get a total volume of 34.5 ml. The volume was made up to 50 ml with sterile double distilled water.

2.4 Steps in DNA isolation

DNA was isolated following the protocol of Doyle and Doyle (1987) with following steps;

1) 5g of fresh leaf tissues were ground to a very fine powder in liquid nitrogen using 7.5 cm mortor and pestle.

2) The powder was transferred to a 50 ml polypropylene centrifuge tube containing 25 ml of pre-warmed (60^0C) DNA extraction buffer.

3) The suspension was incubated at 60^0C for one hour with intermittent swirling.

4) The mixture was emulsified with an equal volume of chloroform: isoamyl alcohol (24:1) for 5 minutes by gentle inversion.

5) The content was centrifuged with Remi C-24 centrifuge at 15,000 rpm for 10 minutes at room temperature.

6) The aqueous phase was transferred to a fresh centrifuge tube with a wide bore pipette and 0.6 volume of chilled iso-propanol was added to it by quick and gentle inversion.

7) The precipitated DNA was spooled out using disposable pipette tip and washed twice with 70% ethanol.

8) The pellet was dried under vaccume and dissolved in 1 ml of T_{10} E_1 buffer.

3. Plant DNA purification

3.1 Chemicals for DNA purification

i. Phenol: chloroform: isoamyl alcohol (25:24:1) mixture.

ii. Chloroform: isoamyl alcohol (24:1) mixture.

iii. 3 M Na-acetate solution (246.1 g) dissolved in double distilled water, volume made up to 1 lit and autoclaved.

iv. Isopropanol.

v. 70% ethanol (dilution made by sterile double distilled water).

vi. Tris-EDTA buffer (T_{10} E_1 i.e. 10 mM Tris and 1 mM EDTA):1.21 g Tris and 0.372 g EDTA was dissolved in double distilled water and pH was adjusted to 8.0 with 1N HCl. Volume made up to 1 lit and autoclaved.

vii. RNase A (T_{10} $NaCl_{15}$ i.e. 10 mM Tris and 15 mM NaCl): 10mg/ml RNase A was dissolved in 10 mM Tris-HCl (pH 7.5) and 15 mM NaCl. The solution was heated at 100^0C for 15 minutes and cooled to room temperature. The stocks were stored at -20^0C.

3.2 Steps in DNA purification

Purification of DNA is essential to remove RNA, proteins and polysaccharides which are considered to be the major contaminants in the DNA precipitates. Inclusion of cTAB in DNA extraction buffer helps elimination of polysaccharides. RNA was removed by RNase treatment and proteins were removed by phenol- chloroform extraction buffer. Following steps were involved in DNA purification:

1. RNase was added to the DNA sample @ mg/500 ml and incubated at 37^0C for 1 hr.

2. After 1 hr equal volume of phenol: chloroform: iso amylalcohol (25:24:1) was added and mixed followed by centrifugation at 10,000 rpm for 5 minutes at room temperature. The aqueous phase was separated into a fresh microfuge tube.

3. The aqueous phase was extracted with chlocroform: iso-amylalcohaol (24:1). The upper aqueous phase was collected and extracted with chloroform: iso amylalcohol (24:1) for the second time.

4. The separated upper aqueous phase was collected after centrifugation and mixed with $1/10^{th}$ volume of 3 M sodium acetate.

5. DNA was precipitated by adding two volumes of chilled absolute alcohol, pelleted by centrifugation at 5,000 rpm for 3 minutes, dried in vacuumed and dissolved in $T_{10}E_1$ buffer.

4. Quantification of DNA

Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel alongside diluted uncut lambda DNA was used as standard. DNA was diluted in $T_{10}E_1$ to a concentration of approximately 12.5 mg/ml for use in PCR analysis.

5. RAPD Analysis

RAPD analysis (William's *et al.,* 1990) was performed using 200 andom primers of Operon series OPA, OPB, OPC, OPM, OPN, OPP, OPS, OPU, OPV and OPW each having 20 series. These were screened using DNA isolated from all the 60 lines (Table 3.4).

6. Setting of PCR reaction

The PCR reactions were carried out in sterile 0.5 mm thin walled PCR tubes obtained from Oxygen Scientific Pvt. Ltd. Union city, CA, USA. Amplification was carried out in 25 ml reaction volume containing the following reagents.

i.	10 x PCR assay buffer	
	(Final conc. 1 x containing 50 mm	2.5ml
	KCl, 10mM tris-HCl, 1.5mM $MgCl_2$)	
ii.	dNTPmix (containing 200 μM each of	
	dATP, dTTP, dGTP and dCTP)	0.5ml
iii.	Primer (20 μg)	1.0ml
iv.	Taq DNA polymerase (stock 3U/μl)	
	(Bangalore Genei Pvt. Ltd., Bangalore, India)	0.5ml
v.	Template DNA (25μg)	2.0ml
vi.	Sterile double distilled water	18ml

The amplification reaction was carried out in a Thermocycler (Perkin Elmer, Model 2400 and Boehringer Mannheim Amplitron II Thermocycler) with the following specifications/cycles:

Cycle 1	Denaturation (94^0C)	5min
	Primer annealing (37^0C)	1min
	Primer extension (72^0C)	2min
Cycle 2-44	Denaturation (92^0C)	1min
	Primer annealing (37^0C)	1min
	Primer extension (72^0C)	2min
Cycle 45	Denaturation (92^0C)	1min
	Primer annealing (37^0C)	1min
	Primer extension (72^0C)	7min

At the end of the run the PCR tubes were taken out and $1/10^{th}$ volume (i.e. 2.5 ml) of 6x loading dye (containing bromophenol blue and xylene cyanol) was added into PCR product.

Table 3.4 Details of the primers and their 5' to 3' base-sequence used in diversity.

S.No.	Primers	Sequence	S.No.	Primers	Sequence
1.	OPA1	CAGGCCCTTC	28.	OPB8	GTCCACACGG
2.	OPA2	TGCCGAGCTG	29.	OPB9	TGGGGGACTC
3.	OPA3	AGTCAGCCAC	30.	OPB10	CTGCTGGGAC
4.	OPA4	AATCGGGCTG	31.	OPB11	GTAGACCCGT
5.	OPA5	AGGGGTCTTG	32.	OPB12	CCTTGACGCA
6.	OPA6	GGTCCCTGAC	33.	OPB13	TTCCCCCGCT
7.	OPA7	GAAACGGGTG	34.	OPB14	TCCGCTCTGG
8.	OPA8	GTGACGTAGG	35.	OPB15	GGAGGGTGTT
9.	OPA9	GGGTAACGCC	36.	OPB16	TTTGCCCGGA
10.	OPA10	GTGATCGCAG	37.	OPB17	AGGGAACGAG
11.	OPA11	CAATCGCCGT	38.	OPB18	CCACAGCAGT
12.	OPA12	TCGGCGATAG	39.	OPB19	ACCCCCGAAG
13.	OPA13	CAGCACCCAC	40.	OPB20	GGACCCTTAC
14.	OPA14	TCTGTGCTGG	41.	OPC1	TTCGAGCCAG
15.	OPA15	TTCCGAACCC	42.	OPC2	GTGAGGCGTC
16.	OPA16	AGCCAGCGAA	43.	OPC3	GGGGGTCTTT
17.	OPA17	GACCGCTTGT	44.	OPC4	CCGCATCTAC
18.	OPA18	AGGTGACCGT	45.	OPC5	GATGACCGCC
19.	OPA19	CAAACGTCGG	46.	OPC6	GAACGGACTC
20.	OPA20	GTTGCGATCC	47.	OPC7	GTCCCGACGA
21.	OPB1	GTTTCGCTCC	48.	OPC8	TGGACCGGTG
22.	OPB2	TGATCCCTGG	49.	OPC9	CTCACCGTCC
23.	OPB3	CATCCCCCTG	50.	OPC10	TGTCTGGGTG
24.	OPB4	GGACTGGAGT	51.	OPC11	AAAGCTGCGG
25.	OPB5	TGCGCCCTTC	52.	OPC12	TGTCATCCCC
26.	OPB6	TGCTCTGCCC	53.	OPC13	AAGCCTCGTC
27.	OPB7	GGTGACGCAG	54.	OPC14	TGCGTGCTTG

Contd.

55.	OPC15	GACGGATCAG	86.	OPN6	GAGACGCACA
56.	OPC16	CACACTCCAG	87.	OPN7	CAGCCCAGAG
57.	OPC17	TTCCCCCCAG	88.	OPN8	ACCTCAGCTC
58.	OPC18	TGAGTGGGTG	89.	OPN9	TGCCGGCTTG
59.	OPC19	GTTGCCAGCC	90.	OPN10	ACAACTGGGG
60.	OPC20	ACTTCGCCAC	91.	OPN11	TCGCCGCAAA
61.	OPM1	GTTGGTGGCT	92.	OPN12	CACAGACACC
62.	OPM2	ACAACGCCTC	93.	OPN13	AGCGTCACTC
63.	OPM3	GGGGGATGAG	94.	OPN14	TCGTGCGGGT
64.	OPM4	GGCGGTTGTC	95.	OPN15	CAGCGACTGT
65.	OPM5	GGGAACGTGT	96.	OPN16	AAGCGACCTG
66.	OPM6	CTGGGCAACT	97.	OPN17	CATTCGGGAG
67.	OPM7	CCGTGACTCA	98.	OPN18	GGTGAGGTCA
68.	OPM8	TCTGTTCCCC	99.	OPN19	GTCCGTACTG
69.	OPM9	GTCTTGCGGA	100.	OPN20	GGTGCTCCGT
70.	OPM10	TCTGGCGCAC	101.	OPP1	GTAGCACTCC
71.	OPM11	GTCCACTGTG	102.	OPP2	TCGGCACGCA
72.	OPM12	GGGACGTTGG	103.	OPP3	CTGATACGCC
73.	OPM13	GGTGGTCAAG	104.	OPP4	GTGTCTCAGG
74.	OPM14	AGGGTCGTTC	105.	OPP5	CCCCGGTAAC
75.	OPM15	GACCTACCAC	106.	OPP6	GTGGGCTGAC
76.	OPM16	GTAACCAGCC	107.	OPP7	GTCCATGCCA
77.	OPM17	TCAGTCCGGG	108.	OPP8	ACATCGCCCA
78.	OPM18	CACCATCCGT	109.	OPP9	GTGGTCCGCA
79.	OPM19	CCTTCAGGCA	110.	OPP10	TCCCGCCTAC
80.	OPM20	AGGTCTTGGG	111.	OPP11	AACGCGTCGG
81.	OPN1	CTCACGTTGG	112.	OPP12	AAGGGCGAGT
82.	OPN2	ACCAGGGGCA	113.	OPP13	GGAGTGCCTC
83.	OPN3	GGTACTCCCC	114.	OPP14	CCAGCCGAAC
84.	OPN4	GACCGACCCA	115.	OPP15	GGAAGCCAAC
85.	OPN5	ACTGAACGCC	116.	OPP16	CCAAGCTGCC

Contd.

117.	OPP17	TGACCCGCCT	148.	OPU8	GGCGAAGGTT
118.	OPP18	GGCTTGGCCT	149.	OPU9	CCACATCGGT
119.	OPP19	GGGAAGGACA	150.	OPU10	ACCTCGGCAC
120.	OPP20	GACCCTAGTC	151.	OPU11	AGACCCAGAG
121.	OPS1	CTACTGCGCT	152.	OPU12	TCACCAGCCA
122.	OPS2	CCTCTGACTG	153.	OPU13	GGCTGGTTCC
123.	OPS3	CAGAGGTCCC	154.	OPU14	TGGGTCCCTC
124.	OPS4	CACCCCCTTG	155.	OPU15	ACGGGCCAGT
125.	OPS5	TTTGGGGCCT	156.	OPU16	CTGCGCTGGA
126.	OPS6	GATACCTCGG	157.	OPU17	ACCTGGGGAC
127.	OPS7	TCCGATGCTG	158.	OPU18	GAGGTCCACA
128.	OPS8	TTCAGGGTGG	159.	OPU19	GTCAGTGCGG
129.	OPS9	TCCTGGTCCC	160.	OPU20	ACAGCCCCCA
130.	OPS10	ACCGTTCCAG	161.	OPV1	TGACGCATGG
131.	OPS11	AGTCGGGTGG	162.	OPV2	AGTCACTCCC
132.	OPS12	CTGGGTGAGT	163.	OPV3	CTCCCTGCAA
133.	OPS13	GTCGTTCCTG	164.	OPV4	CCCCTCACGA
134.	OPS14	AAAGGGGTCC	183.	OPW3	GTCCGGAGTG
135.	OPS15	CAGTTCACGG	184.	OPW4	CAGAAGCGGA
136.	OPS16	AGGGGGTTCC	185.	OPW5	GGCGGATAAG
137.	OPS17	TGGGGACCAC	186.	OPW6	AGGCCCGATG
138.	OPS18	CTGGCGAACT	187.	OPW7	CTGGACGTCA
139.	OPS19	GAGTCAGCAG	188.	OPW8	GACTGCCTCT
140.	OPS20	TCTGGACGGA	189.	OPW9	GTGACCGAGT
141.	OPU1	ACGGACGTCA	190.	OPW10	TCGCATCCCT
142.	OPU2	CTGAGGTCTC	191.	OPW11	CTGATGCGTG
143.	OPU3	CTATGCCGAC	192.	OPW12	TGGGCAGAAG
144.	OPU4	ACCTTCGGAC	193.	OPW13	CACAGCGACA
145.	OPU5	TTGGCGGCCT	194.	OPW14	CTGCTGAGCA
146.	OPU6	ACCTTTGCGG	195.	OPW15	ACACCGGAAC
147.	OPU7	CCTGCTCATC	196.	OPW16	CAGCCTACCA

Contd.

197.	OPW17	GTCCTGGGTT	199.	OPW19	CAAAGCGCTC
198.	OPW18	TTCAGGGCAC	200.	OPW20	TGTGGCAGCA

7. Agarose gel electrophoresis

The amplification products in RAPD analysis were separated by electrophoresis in 1.5% agarose gel containg ethidium bromide using 1xTAE buffer (pH8.0) 1xTAE buffer was made by diluting the 50xTAE buffer 50 times (50xTAE was prepared by dissolving 242g tris base, 57.1 ml glacial acetic acid and 100 ml of 0.5M EDTA in distilled water and volume was made upto 1 lit. Separation was carried out by applying constant voltage 5 V/cm for 3 hrs in Biorad –200V power-pack. The size of amplified fragments was determined by using size standard (100 bp DNA ladder mix, MBI fragment, Lithuania). DNA fragments were visualized under UV light and photographed using Jeldoc photographic system for permanent records.

8. Scoring of band and data analysis

Each amplification product was considered as RAPD marker (unit character) and scored across all the samples as discrete variable. Data were entered using a matrix in which all observed bands or characters were listed. The RAPD pattern of each isolate were evaluated assigning 'Character state' to all bands that could be observed in gel with certain primers. The character state 'j' was given if this band could be reproducibly detected in all the RAPD analysis with the isolate primer combination. The character state 'O' was assigned if it was lacking or it was not possible to determine it with certainty. The data matrix thus generated was used to calculate Jaccard's Similarity Coefficient for each pair-wise comparisons. The coefficient was calculated following Jaccard (1908):

Similarity coefficient=a/n

Where,

a=number of matching bands for each pair of comparisons.

b=total number of bands in two samples observed.

The similarity coefficient were subjected to unweighted pair-group method on arithmetic average (UPGMA) method of cluster analysis to group the isolates based on their overall similarities. NTSYS-pc version 1.6 software (Rohlf, 1990) was used for cluster analysis.

3.4 Analysis of the data

3.4.1 Qualitative characters

The observations on qualitative characters such as growth habit, plant pubescence, pigmentation, tendril formation, leaf size, leaf shape, leaf colour, stipule size, peduncle length, flower and seed coat colour which were recorded on line basis were used grouping the germplasm line and varieties.

3.4.2 Quantitative characters

The data collected on the 12 quantitative characters were subjected to the following statistical analysis:

1. Analysis of variance
2. Mean, range and variability
3. Genetic divergence
4. Coefficient of correlation
5. Path analysis
6. Heritability
7. Genetic advance
8. Similarity coefficient (index)/dendrogram

1. Analysis of variance

The analysis of the experimental design was based on the following model:

$P_{ijk} = \mu + v_{ij} + r_k + e_{ijk}$

(i,j = 1--------t ; k =1----------r)

I#j

Where,

P_{ijk} = the phenotype of ijk^{th} observation

μ = the population mean

v_{ij} = progeny effect

r_k = the effect of k^{th} replication

e_{ijk} = the error of ijk^{th} observation.

On this model, the data obtained on different characters from 100 germplasm lines including released varieties and checks were first subjected to analysis of augmented randomized complete block design as per the procedure laid down by Federer and Raghavarao (1975). The mean values of each character in each line/replication were then used in this analysis. The skeleton of analysis has been given below as follows:

Table 3.5 Skeleton of analysis of variance of the experiment

Source	d.f.	MSS	'F'-value
Replication	(r-1)	r_{mss}	r_{mss}/e_{mss}
Lines	(l-1)	l_{mss}	l_{mss}/e_{mss}
Checks	(c-1)	c_{mss}	c_{mss}/e_{mss}
Unreplicated lines	(url -1)	url $_{mss}/e_{mss}$	url $_{mss}/e_{mss}$
Unreplicated lines Vs checks	1	url vs. c_{mss}	url vs c_{mss}/e_{mss}
Error	(r-1)(c-1)	e_{mss}	------

Where, r = replication, l = lines, c = Checks, url = Unreplicated lines, e = error, mss = mean sum of squares.

2. Mean, range and variability

(a) **Mean**: The mean of each character in each population was worked out by taking the average of number of observations recorded.

(b) **Range**: The range, which is also a measure of variability, was recorded by taking the lowest and highest values of the observation in each population for each character.

(c) **Variability**: The variability at genotypic, phenotypic and environmental levels or calculated through mean sum of squares (MSS) of the analysis of variance of the experiment following the formula given below:

i. $\text{Genotypic variance (Vg)} = \dfrac{\text{Urlmss - emss}}{\text{r}}$

ii. Phenotypic variance (Vp) = Genotypic variance + emss

iii. Error variance (Ve) = emss

(d) **Coefficient of variability**: In order to compare different characters in respect of variability, the coefficient of variability was estimated by using the following formula.

(i) $\text{Genotypic coefficient of variability} = \dfrac{\text{Vg}}{\bar{\text{X}}} \times 100$

(ii) $\text{Phenotypic coefficient of variability} = \dfrac{\text{Vp}}{\bar{\text{X}}} \times 100$

(iii) $\text{Environmental coefficient of variability} = \dfrac{\text{Ve}}{\bar{\text{X}}} \times 100$

3. Genetic divergence

For working our the genetic divergence among 100 germplasm lines, the procedure suggested by Rao (1952) was followed. The related statistics, such as intra and inter cluster divergences, formation of cluster on the basis of closeness in terms of divergence and performance of individual character across the cluster formed were also estimated.

4. Correlation coefficient

Correlation coefficient was calculated by the formula suggested by Robinson *et al.,* (1951) using the variance and covariance components. The calculations were based on mean value of the characters.

$$\text{Correlation coefficient (r)} = \frac{\varepsilon (X - \bar{X}).(Y - \bar{Y})}{\sqrt{\varepsilon (X - \bar{X})^2 . \varepsilon (Y - \bar{Y})^2}}$$

Where,

X and Y are the two characters for which correlation coefficient is being worked out.

i) Phenotypic correlation coefficient (rp)

$$\sigma^2 \text{ph } 1.2 = \frac{\sigma^2 \text{ph } 1.2}{\sqrt{\sigma^2 \text{ph}_1 \times \sigma^2 \text{ph}^2}}$$

Where σ^2 ph 1.2 = phenotypic covariance between two trait (1 and 2)

σ^2 ph_1 = phenotypic variance of first trait

σ ph_2 = phenotypic variance of the second trait

ii) Genotypic correlation coefficient (rg)

Genotypic covariance is obtained by deducting error covariance from phenotypic covariance and than dividing that by number of replication after that (rg) is calculated as follows:

$$rg_{1.2} = \frac{\sigma g_{1.2}}{\sqrt{\sigma^2 g_1 \times \sigma^2 g_2}}$$

Where,

$\sigma g_{1.2}$ = Genotypic covariance between two trait

$\sigma^2 g_1$ = Genotypic variance of first trait

$\sigma^2 g_2$ = Genotypic variance of second trait

iii) Environmental correlation coefficient (re)

$$re_{1.2} = \frac{\sigma^e_{1.2}}{\sqrt{\sigma^2 e_1 \times \sigma^2 e_2}}$$

Where

$\sigma^e_{1.2}$ = Error covariance between two trait (1 and 2)

$\sigma^2 e_1$ = Error variance of first traits

$\sigma^2 e_2$ = Error variance of second traits

Phenotypic and environmental correlation coefficient were compared against r-value given by Edward (1972) at (t-2) and (r-1) (t-1)-1 degrees of freedom, respectively, at p = 0.05 and p = 0.01 levels to test their significance.

5. Path analysis

Path coefficient is the ratio of standard deviation of effect to the total standard deviation when all are causes are constant, except one in question, the variability of which was kept unchanged, was obtained by he simultaneous solution of the following equations with the help of matrix algebras, which expresses the basic relationship between correlation and path coefficients.

1. $r_{17} = p_{17} + r_{12}.p_{27} + r_{13}.p_{37} + r_{14}.p_{47} + r_{15}.p_{57}$
2. $r_{27} = p_{27} + r_{27}.p_{17} + r_{23}.p_{37} + r_{24}.p_{47} + r_{25}.p_{57} + r_{26}.p_{67} + r_{28}.p_{87}$
3. $r_{37} = p_{37} + r_{31}.p_{17} + r_{32}.p_{27} + r_{37}.p_{47} + r_{35}.p_{57} + r_{36}.p_{67} + r_{38}.p_{87}$
4. $r_{47} = p_{47} + r_{41}.p_{17} + r_{42}.p_{27} + r_{43}.p_{47} + r_{45}.p_{57} + r_{46}.p_{67} + r_{48}.p_{87}$
5. $r_{57} = p_{57} + r_{51}.p_{17} + r_{52}.p_{27} + r_{53}.p_{37} + r_{54}.p_{47} + r_{54}.p_{67} + r_{58}.p_{87}$
6. $r_{67} = p_{67} + r_{61}.p_{17} + r_{62}.p_{27} + r_{63}.p_{37} + r_{64}.p_{47} + r_{65}.p_{57} + r_{58}.p_{87}$
7. $r_{87} = p_{87} + r_{81}.p_{17} + r_{82}.p_{27} + r_{83}.p_{37} + r_{84}.p_{47} + r_{85}.p_{57} + r_{86}.p_{6}$

Where,

p = path coefficient

r = correlation coefficient

The residual effect was calculated by following formula.

$R = 1 - (piy.riy)$

Where

R = Residual effect

piy = path coefficient of $(i)^{th}$ character with yield

Riy = Genotypic correlation of $(i)^{th}$ character with yield

The indirect effect of a character via another causal factor was obtained by multiplying the genotypic correlation coefficient between the two with direct effect (i.e. but coefficient) of the latter upon effect.

6. Estimation of heritability

The heritability in broad sense was estimated using the formula of Burton and DeVane (1953).

$$\text{Heritability} = \frac{Vg}{Vp} \times 100$$

Where,

Vg = Genotypic variance

Vp = Phenotypic variance

7. Genetic advance

The genetic advance was worked out by the formula proposed by Robinson *et al.*, (1949) as follows:

$$G.A = K(h^{\wedge 2})\ (\sigma^2 ph)$$

$$G.A \text{ over mean} = \frac{GA}{\overline{X}} \times 100$$

Where,

G.A = estimate of genetic advance

K = selection differential at 5% selection intensity, i.e. 2.06

$\sigma^2 ph$ = phenotypic standard deviation

$h^{\wedge 2}$ = estimate of heritability coefficient.

$\overline{X}$ = mean of the character.

EXPERIMENTAL FINDINGS

In the present investigation "**Characterization of lentil germplasm through morphological and metric trait and molecular markers**" a set of 100 germplasm lines, including varieties and checks, were raised following the augmented randomized complete block design during *rabi* 2003-2004 and observations on fourteen morphological and qualitative character viz. growth habit, plant pubescence, pigmentation, tendril formation, leaf size, leaf shape, leaf colour, stipule size, peduncle length, flower colour, testa colour, testa pattern, seed shape, cotyledon colour and twelve metric traits viz. days to flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, number of flowers per peduncle, number of pods/plant, number of seeds per pod, 100-seed weight, grain yield, biomass score, harvest index were recorded. A set of 100 germplasm lines, including varieties and checks, out of 100 test entries were raised during *rabi* 2004-2005, following the same layout, methods and package of practices as were adopted during the first year of experimentation and the observations on molecular markers were recorded. The data recorded on the different characters were subjected to appropriate statistical analysis as per recommended procedure and the results are being presented under the following heads:

4.1. Morphological markers/dendrogram

4.2. Quantitative characters

4.2.1 Analysis of variance

4.2.2 Mean, range, variability.

4.2.3 Genetic divergence.

4.2.4 Coefficient of correlation.

4.2.5 Path coefficient analysis.

4.2.6 Heritability.

4.2.7 Genetic advance.

4.3. Molecular marker/dendrogram

4.1 Morphological markers

A set of 100 germplasm lines including varieties and checks, were categorized in terms of morphological and qualitative traits. Since these characters are not influenced by the environmental factors, no statistical analysis for neutralizing or separating the error component was made. The description of each of these lines in terms of these morphological and qualitative characters has been made and the same has been presented in (Table 4.1). As described under the chapter "Materials and methods" there were fourteen morphological and qualitative traits under study.

From (Table 4.2) it can be seen that 100 germplasm lines on which morphological and qualitative characters have been recorded covered only 14 characters and 44 categories. All the character described below in respect of different categories. Some of the character can be seen in (Fig. 1-4) and (Fig. 5) for seed coat colour.

Table 4.1 : Details of various distinguishable qualitative characters in 100 accessions of lentil.

Accession	Growth Habit.	Plant Pubescence	Pigmentation		Tendril Formation	Leaf size	Leaf shape	Leaf colour	Stipule size	Peduncle length	Flower colour	Testa colour	Testa pattern	Seed shape	Cotyledon colour
			Leaf	Stem											
Sehore74-3	Prostrate	Present	Present	Present	Present	Medium	Oval	Green	Short	Short	Purple	Green	Mottled	Spherical	Orange
LH90-57	Prostrate	Absent	Absent	Absent	Absent	Medium	Oval	Light green	Long	Long	Light purple	Brown	Mottled	Flat	Yellow
L-830	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
JLS-1	Prostrate	Present	Present	Present	Present	Medium	Oval	Green	Short	Short	Darkpurple	Brown	Mottled	Spherical	Orange
LC93-5-3-3-1	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Short	Short	Light purple	Black	Mottled	Spherical	Orange
L-4149	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Long	Long	Light purple	Green	Mottled	Flat	Orange
E-153	Erect	Present	Present	Present	Present	Broad	Acute	Green	Short	Short	Light purple	Green	Mottled	Flat	Orange
L-1304	Prostrate	Present	Present	Present	Present	Medium	Oval	Dark green	Short	Long	Purple	Gray	Mottled	Spherical	Green
L-4147	Prostrate	Present	Present	Present	Absent	Medium	Acute	Dark green	Short	Short	Light purple	Gray	Mottled	Spherical	Orange
L-4378	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Long	Purple	Green	Mottled	Spherical	Orange
L-4384	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Long	Light purple	Brown	Mottled	Spherical	Green
L-435	Prostrate	Absent	Present	Absent	Present	Medium	Acute	Green	Short	Short	Purple	Black	Mottled	Spherical	Orange
SKL-259	Prostrate	Present	Present	Present	Present	Narrow	Acute	Green	Short	Long	Purple	Gray	Mottled	Spherical	Orange
10-2-B-2	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Short	Purple	Green	Mottled	Spherical	Brown
L-3685	Prostrate	Present	Present	Absent	Present	Narrow	Acute	Light green	Short	Long	Purple	Black	Mottled	Spherical	Orange
L-4387	Prostrate	Present	Present	Present	Present	Narrow	Acute	Green	Short	Long	Purple	Green	Mottled	Spherical	Light green
LC74-1-5-1	Prostrate	Present	Absent	Present	Present	Narrow	Acute	Dark green	Short	Short	Purple	Green	Mottled	Flat	Light green
Pusa-4	Prostrate	Present	Present	Present	Absent	Narrow	Acute	Dark green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
PL-639	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Short	Long	Purple	Gray	Mottled	Spherical	Orange
L-4603	Erect	Absent	Absent	Absent	Present	Narrow	Acute	Light green	Short	Short	White	Brown	Mottled	Spherical	Yellow
L-4602	Prostrate	Absent	Present	Present	Absent	Broad	Acute	Green	Long	Short	Light purple	Green	Mottled	Flat	Yellow
Fasciated mutant	Prostrate	Present	Present	Present	Absent	Medium	Oval	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
Dwarf mutant	Erect	Absent	Present	Present	Present	Narrow	Oval	Light green	Short	Short	Light purple	Brown	Mottled	Spherical	Orange
8-1	Prostrate	Absent	Present	Present	Present	Medium	Acute	Green	Short	Long	Light purple	Gray	Mottled	Spherical	Green

Contd.

Globe meerut	Prostrate	Present	Present	Present	Absent	Medium	Acute	Green	Short	Long	Light purple	Brown	Mottled	Flat	Orange
10-3Y-26 normal	Prostrate	Present	Absent	Present	Absent	Medium	Acute	Green	Long	Short	Light purple	Brown	Mottled	Spherical	Yellow
19-B-5 globe	Prostrate	Present	Present	Present	Absent	Medium	Acute	Green	Short	Short	Light purple	Green	Mottled	Flat	Yellow
19-B-5 normal	Prostrate	Absent	Present	Present	Absent	Medium	Acute	Green	Short	Short	Light purple	Gray	Mottled	Spherical	Green
L-4605	Prostrate	Present	Present	Present	Present	Broad	Oval	Light green	Long	Long	Purple	Gray	Mottled	Spherical	Yellow
L-6183	Prostrate	Absent	Present	Present	Present	Medium	Oval	Dark green	Short	Long	Light purple	Gray	Mottled	Flat	Green
PL-406	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Long	Purple	Brown	Mottled	Spherical	Orange
LC68-17-4-0	Erect	Absent	Absent	Present	Present	Narrow	Acute	Light green	Short	Long	Purple	Gray	Mottled	Spherical	Orange
EC383090	Erect	Absent	Present	Present	Absent	Broad	Oval	Light green	Short	Short	White	Tan	Mottled	Flat	Yellow
EC383087	Erect	Absent	Absent	Absent	Present	Broad	Oval	Light green	Short	Short	White	Tan	Non-mottled	Flat	Yellow
P22211	Erect	Absent	Absent	Absent	Present	Narrow	Oval	Light green	Short	Short	White	Tan	Non-mottled	Flat	Orange
P33159	Erect	Absent	Present	Present	Present	Narrow	Acute	Light green	Short	Short	White	Tan	Non-mottled	Flat	Orange
14-1-Y-50	Erect	Absent	Present	Present	Present	Narrow	Acute	Light green	Short	Long	White	Brown	Non-mottled	Flat	Yellow
L4076	Prostrate	Present	Present	Present	Present	Broad	Oval	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
L97/454-1	Erect	Absent	Present	Present	Absent	Narrow	Acute	Light green	Short	Long	Purple	Brown	Non-mottled	Flat	Yellow
MC-6	Erect	Present	Absent	Present	Present	Broad	Oval	Light green	Short	Long	White	Tan	Mottled	Flat	Yellow
MC-1	Prostrate	Absent	Present	Absent	Present	Medium	Oval	Dark green	Short	Long	White	Brown	Non-mottled	Spherical	Yellow
25-26	Prostrate	Present	Absent	Present	Present	Medium	Acute	Green	Short	Long	Light purple	Green	Mottled	Flat	Orange
LC68-17-3-5	Erect	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Short	White	Tan	Mottled	Flat	Yellow
8-3	Prostrate	Absent	Present	Present	Present	Medium	Acute	Green	Short	Short	Light purple	Yellow	Mottled	Flat	Orange
ICARDA PR.	Prostrate	Absent	Present	Present	Absent	Medium	Acute	Dark green	Short	Long	Purple	Brown	Mottled	Spherical	Yellow
EC383084	Erect	Absent	Absent	Present	Present	Broad	Oval	Light green	Long	Short	White	Tan	Non-mottled	Flat	Orange
P22127	Erect	Absent	Absent	Present	Present	Broad	Oval	Light green	Long	Short	White	Tan	Non-mottled	Flat	Yellow
RL-1	Erect	Absent	Absent	Present	Present	Medium	Acute	Green	Short	Short	White	Green	Non-mottled	Flat	Yellow
P22107	Erect	Absent	Absent	Absent	Present	Medium	Acute	Light green	Short	Short	White	Black	Non-mottled	Flat	Yellow
L-5258	Prostrate	Absent	Absent	Absent	Present	Narrow	Acute	Light green	Short	Short	White	Brown	Non-mottled	Flat	Orange
PKVL-1	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
P22115	Erect	Absent	Absent	Absent	Present	Narrow	Acute	Light green	Short	Short	White	Tan	Non-mottled	oval	Orange

Contd.

10-3Y-26 globe	Erect	Present	Present	Present	Present	Broad	Oval	Dark green	Long	Short	Purple	Tan	Mottled	globe	Orange
FLIP-96-51	Prostrate	Present	Present	Present	Absent	Narrow	Acute	Green	Short	Long	White	Brown	Non-mottled	Flat	Yellow
PL-5	Prostrate	Present	Present	Present	Absent	Medium	Acute	green	Short	Short	White	Dark green	Mottled	Flat	Yellow
DPL-21	Prostrate	Present	Present	Present	Absent	Medium	Acute	Green	Short	Short	Purple	Green	Mottled	Spherical	Yellow
DPL-15	Prostrate	Present	Present	Present	Absent	Medium	Oval	Green	Short	Short	Light purple	Gray	Mottled	Spherical	Orange
DPL-62	Prostrate	Absent	Present	Present	Absent	Medium	Acute	Green	Short	Long	Dark purple	Black	Mottled	Flat	Orange
IPL-81	Present	Present	Absent	Absent	Absent	Medium	Oval	Green	Short	Long	Purple	Brown	Mottled	Spherical	Orange
L5120	Present	Absent	Present	Present	Absent	Broad	Acute	Green	Long	Short	White	Green	Mottled	Flat	Yellow
L-5214	Erect	Absent	Present	Present	Present	Medium	Oval	Light green	Long	Long	White	Brown	Non-Mottled	Flat	Orange
L-7481	Prostrate	Absent	Absent	Absent	Present	Narrow	Acute	Green	Short	Short	White	Brown	Mottled	Spherical	Yellow
L-5214 A	Erect	Absent	Present	Present	Present	Medium	Acute	Light green	Long	Long	Light purple	Brown	Mottled	Flat	Orange
L1195-1770	Prostrate	Present	Present	Present	Present	Broad	Oval	Green	Short	Short	Purple	Brown	Mottled	Spherical	Yellow
L-5214 B	Erect	Absent	Present	Present	Present	Medium	Oval	Light green	Long	Long	Light purple	Brown	Non-mottled	Flat	Yellow
Precoz	Erect	Absent	Present	Present	Present	Broad	Oval	Light green	Long	Long	White	Tan	Non-mottled	Flat	Yellow
L5115	Erect	Present	Present	Present	Present	Medium	Acute	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
L6171	Prostrate	Present	Present	Present	Present	Medium	Acute	Dark green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
L4636	Erect	Present	Present	Present	Present	Medium	Acute	Light green	Short	Short	Light purple	Buff	Non-mottled	Flat	Orange
L5227	Erect	Present	Present	Absent	Present	Narrow	Acute	Light green	Short	Short	Light purple	Gray	Mottled	Spherical	Orange
L5086	Erect	Present	Absent	Present	Present	Medium	Acute	Light green	Short	Long	Purple	Brown	Mottled	Spherical	Orange
P3101	Prostrate	Present	Present	Present	Present	Medium	Acute	Dark green	Short	Long	Purple	Tan	Non-mottled	Flat	Orange
P19132	Prostrate	Present	Present	Present	Absent	Broad	Oval	Light green	Short	Long	Purple	Brown	Non-mottled	Flat	Yellow
L7476	Prostrate	Absent	Present	Present	Present	Medium	Oval	Light green	Long	Long	Purple	Brown	Non-mottled	Spherical	Yellow
L7477	Prostrate	Absent	Absent	Absent	Present	Medium	Acute	Light green	Short	Short	Purple	Brown	Non-mottled	Spherical	Yellow
L7462	Prostrate	Absent	Absent	Absent	Present	Narrow	Acute	Light green	Short	Short	Purple	Gray	Non-mottled	Spherical	Yellow
L5228	Erect	Present	Absent	Absent	Present	Medium	Acute	Green	Short	Short	Purple	Buff	Non-mottled	Spherical	Orange
L3136	Prostrate	Present	Present	Present	Present	Narrow	Acute	Light green	Short	Short	Purple	Green	Mottled	Spherical	Yellow
L5084	Prostrate	Present	Absent	Absent	Present	Narrow	Acute	Dark green	Short	Short	Purple	Brown	Non-mottled	Spherical	Green
6/7	Prostrate	Present	Absent	Absent	Absent	Narrow	Acute	Green	Short	Long	Light purple	Brown	Mottled	Spherical	Orange

Contd.

Rajendra II	Prostrate	Present	Present	Present	present	Medium	Acute	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
L7474	Prostrate	Absent	Absent	Absent	Present	Medium	Acute	Light green	Short	Long	Purple	Gray	Mottled	Spherical	Yellow
L4592	Erect	Present	Absent	Absent	Present	Narrow	Acute	Green	Short	Short	Light purple	Green	Non-mottled	Spherical	Orange
6-59	Prostrate	Present	Present	Present	Present	Narrow	Acute	Green	Short	Short	Purple	Green	Mottled	Spherical	Yellow
L7461	Prostrate	Absent	Present	Present	Absent	Medium	Acute	Light green	Short	Long	Purple	Buff	Mottled	Spherical	Orange
L7463	Prostrate	Present	Absent	Absent	Present	Medium	Acute	Light green	Long	Short	Purple	Buff	Mottled	Flat	Orange
L7473	Prostrate	Present	Present	Present	Present	Broad	Oval	Light green	Short	Short	Purple	Buff	Non-mottled	Flat	Orange
L7459	Prostrate	Present	Present	Present	Present	Broad	Oval	Light green	Short	Long	Purple	Buff	Mottled	Spherical	Orange
P3107	Prostrate	Present	Present	Present	Present	Broad	Oval	Light green	Short	Long	Light purple	Buff	Non-mottled	Spherical	Orange
K75	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
P2180	Erect	Present	Present	Absent	Present	Broad	Acute	Light green	Short	Long	Purple	Buff	Non-mottled	Spherical	Orange
P2215	Erect	Absent	Present	Present	Present	Broad	Oval	Light green	Short	Long	Light purple	Buff	Mottled	Spherical	Orange
L7354	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Long	Short	Purple	Gray	Mottled	Spherical	Yellow
L4614	Prostrate	Present	Present	Present	Present	Narrow	Acute	Dark green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
L6173	Prostrate	Present	Present	Absent	Present	Medium	Acute	Green	Short	Short	Purple	Brown	Mottled	Spherical	Orange
P19235	Prostrate	Absent	Absent	Present	Absent	Broad	Oval	Light green	Short	Short	Darkpurple	Buff	Non-mottled	Spherical	Yellow
P2228	Prostrate	Absent	Present	Absent	Absent	Broad	Oval	Light green	Short	Short	Darkpurple	Buff	Non-mottled	Flat	Yellow
P3111	Prostrate	Absent	Present	Present	Absent	Narrow	Oval	Light green	Short	Long	Light purple	Buff	Non-mottled	Flat	Yellow
L7358	Prostrate	Present	Present	Present	Present	Medium	Acute	Green	Long	Short	Purple	Gray	Mottled	Spherical	Yellow
L7479	Prostrate	Present	Absent	Present	Present	Broad	Oval	Light green	Short	Long	Darkpurple	Buff	Non-mottled	Flat	Yellow

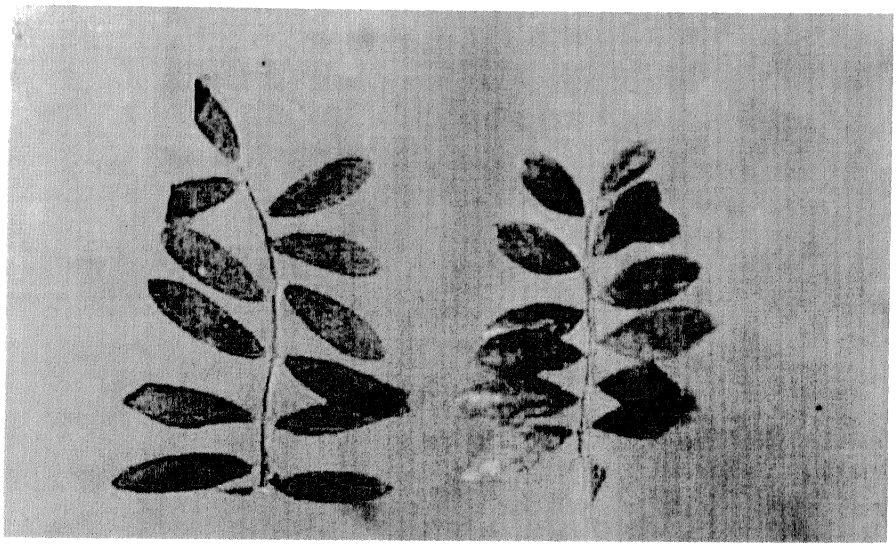

Fig 1. Non- Pigmented (left) and pigmented leaf (right) in lentil

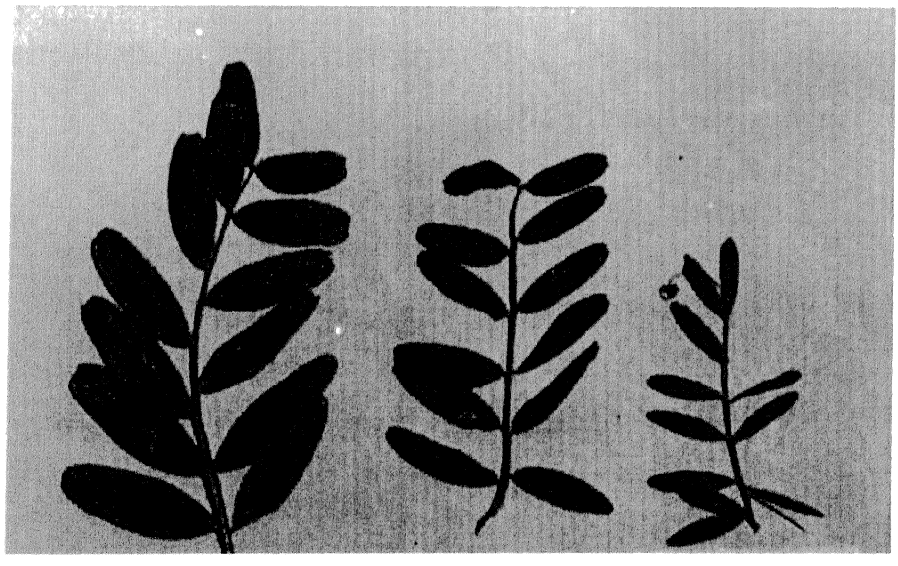

Fig. 2. Leaf size in lentil: broad (left), medium (middle) and narrow (right)

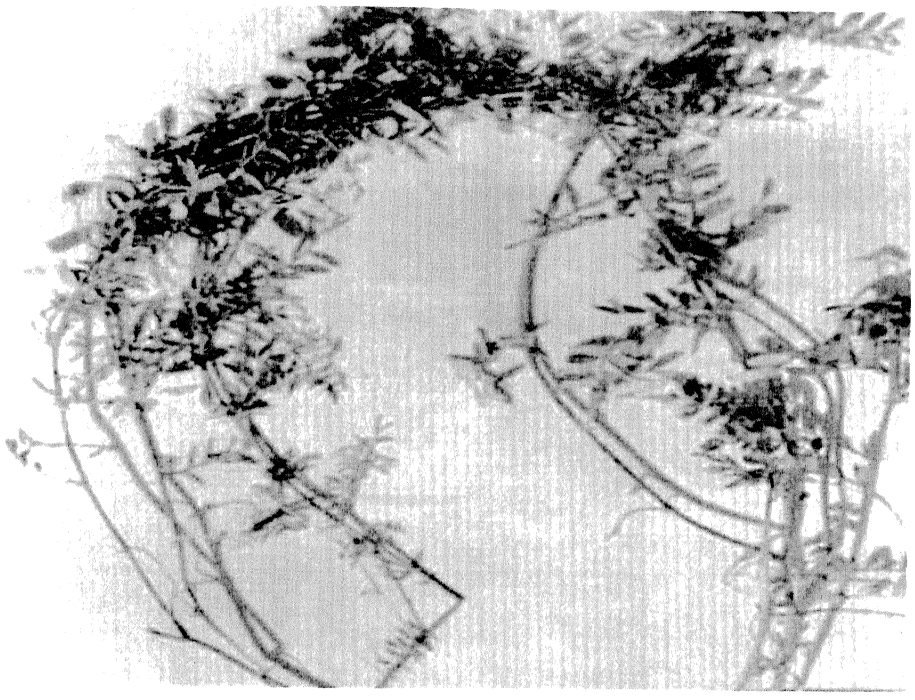

Fig. 3. Non-pigmented (left) and pigmented stem (right) in lentil

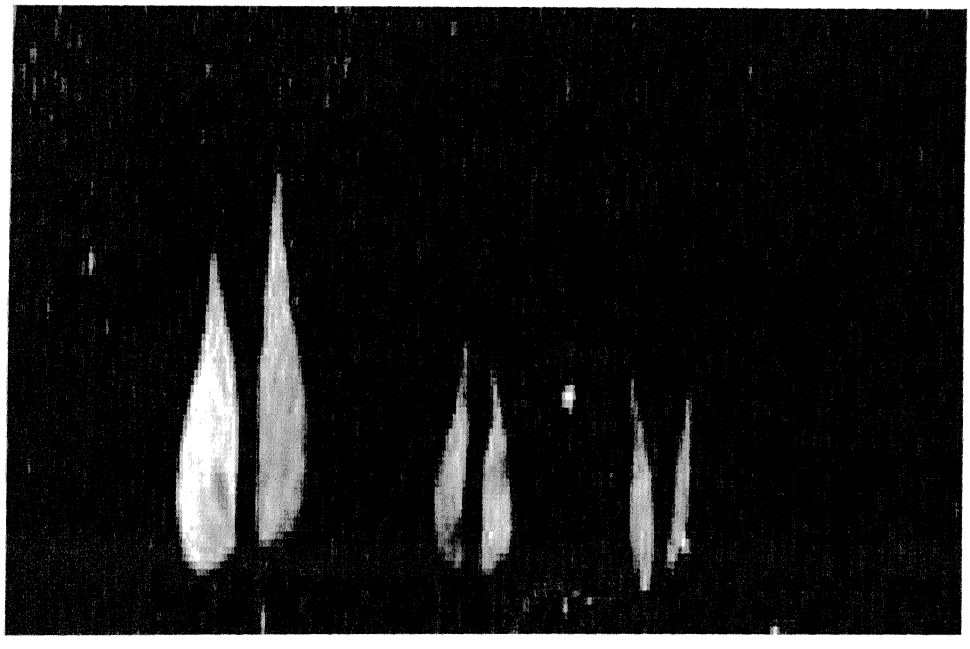

Fig. 4. Stipule size in lentil: large (left), medium (middle) and small (right)

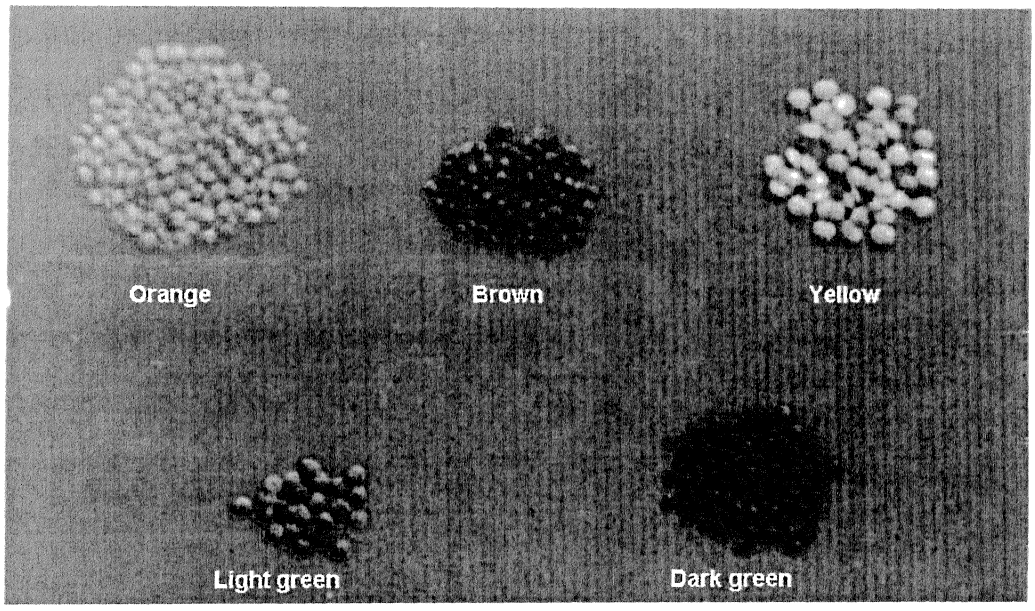

Fig 5. Variability for cotyledon colour in lentil.

1. Growth habit

Two types of growth habit namely, prostrate and erect were observed, seventy genotypes were prostrate and 30 were erect out of 100 germplasm lines and varieties.

2. Plant pubescence

In this case two types of plant pubescence involving pubescent and non-pubescent could be recorded, fifty nine genotypes were pubescent and forty one genotypes were non-pubescent out of 100 lines and varieties under investigation.

3. Leaf pigmentation

Two types of plants namely pigmented and non-pigmented were observed. Out of 100 germplasm studied, seventy-one genotypes were pigmented whereas twenty-nine genotypes were non-pigmented.

4. Stem pigmentation

In the present experiment, two types of stem viz., pigmented and non-pigmented were observed. Seventy-five genotypes were pigmented and twenty-five genotypes were non-pigmented out of 100 lines and varieties used in the study.

5. Tendril formation

Plants were observed in the present study, However, tendrilled plant and non-tendriled seventy five genotypes were tendrilled and twenty five was non tendriled.

6. Leaf size

Three types of leaves viz., broad, medium and narrow were observed among the genotypes. Twenty-one genotypes were broad, leaved, forty-seven genotypes were medium and thirty-two were having narrow leaves out of 100 lines and varieties tested.

7. Leaf shape

It was observed that only two types of leaf shape viz. oval and acute were available in the materials studied thirty-two genotypes were oval and sixty eight genotypes were acute out of 100 accessions use in the study.

8. Leaf colour

Found three different types of leaf colours namely green, light green and dark green. Thirty eight genotypes were green, forty three genotypes were light green and nineteen were having dark green leaves.

9. Stipule size

It was observed that two types of stipules were present in the materials under study. Seventeen genotypes belonged to long and eighty three genotypes were having short stipules.

10. Peduncle length

It was noted that forty one genotypes were long and fifty nine genotypes were having short peduncle out of 100 lines and varieties, included in the study.

Table 4.2 Distribution of 100 germplasm lines and varieties of lentil.

S.No	Character	Categories	Number of genotypes	Lines and varieties
1.	Growth habit	Prostrate	70	L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L21, L22, L23, L24, L25, L26, L27, L28, L29, L30, L31, L32, L33, L34, L35, L36, L37, L38, L39, L40, L41, L42, L43, L44, L45, L46, L47, L48, L49, L50, L51, L52, L53, L54, L55, L56, L57, L58, L59, L60, L61, L62, L63, L64, L65, L66, L67, L68, L69, L70, L71, L72, L73, L74, L75, L76, L77, L78, L79, L80, L81, L82, L83, L84, L85, L86, L87, L88, L89, L90, L91, L92, L93, L94, L95, L96, L97, L98, L99, L100.
		Erect	30	L7, L20, L23, L32, L33, L34, L35, L36, L37, L39, L40, L43, L46, L47, L48, L49, L52, L53, L61, L63, L65, L66, L67, L69, L70, L71, L77, L83, L91, L92.
2.	Plant pubescence	Pubescent	59	L1, L3, L4, L5, L6, L7, L8, L9, L10, L11, L13, L14, L15, L16, L17, L18, L19, L22, L25, L26, L27, L29, L31, L40, L42, L43, L51, L53, L54, L55, L56, L57, L59, L64, L67, L68, L69, L70, L71, L72, L73, L77, L78, L79, L80, L81, L83, L84, L86, L87, L88, L89, L90, L91, L93, L94, L95, L99, L100.
		Non-Pubescent	41	L2, L12, L20, L21, L23, L24, L28, L29, L32, L33, L34, L35, L36, L37, L38, L39, L41, L44, L45, L46, L47, L48, L49, L50, L52, L58, L60, L1, L61, L62, L63, L65, L66, L71, L75, L76, L82, L85, L92, L96, L97, L98.
3.	Leaf pigmentation	Pigmented	71	L1, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L21, L22, L23, L24, L25, L27, L28, L29, L30, L31, L33, L36, L37, L38, L39, L41, L43, L44, L45, L51, L53, L54, L55, L56, L57, L58, L60, L61, L63, L65, L66, L67, L68, L69, L70, L72, L73, L74, L78, L81, L84, L85, L87, L88, L89, L90, L91, L92, L93, L94, L95, L97, L98, L99.
		Non-pigmented	29	L2, L17, L20, L26, L32, L34, L35, L40, L42, L46, L47, L48, L49, L50, L52, L59, L62, L64, L71, L75, L76, L77, L79, L80, L82, L83, L86, L96, L100.

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4.	Stem pigmentation	Pigmented	75	L1, L3, L4, L5, L6, L7, L8, L9, L10, L11, L13, L14, L16, L17, L18, L19, L21, L22, L23, L24, L25, L26, L27, L28, L29, L30, L31, L32, L33, L36, L37, L38, L39, L40, L42, L43, L44, L45, L47, L48, L51, L53, L54, L55, L56, L57, L58, L60, L61, L63, L65, L66, L67, L68, L69, L71, L72, L73, L74, L78, L81, L84, L85, L87, L88, L89, L90, L92, L93, L94, L96, L98, L99, L100.
		Non-Pigmented	25	L2, L12, L15, L20, L34, L35, L41, L49, L50, L52, L59, L62, L64, L70, L75, L76, L77, L79, L80, L82, L83, L86, L91, L95, L97.
5.	Tendril formation	Tendrilled	75	L1, L3, L4, L5, L6, L7, L8, L10, L11, L12, L13, L14, L15, L16, L17, L19, L20, L23, L24, L29, L30, L31, L32, L34, L35, L36, L37, L38, L40, L41, L42, L43, L44, L46, L47, L48, L49, L50, L51, L52, L53, L61, L62, L63, L64, L65, L66, L67, L68, L69, L70, L71, L72, L74, L75, L76, L77, L78, L79, L81, L82, L83, L84, L86, L87, L88, L89, L90, L91, L92, L93, L94, L95, L99, L100.
		Non-tendrilled	25	L2, L9, L18, L21, L22, L25, L26, L27, L28, L33, L39, L45, L54, L55, L56, L57, L58, L59, L60, L73, L80, L85, L96, L97, L98.
6.	Leaf-size	Broad	21	L7, L21, L29, L33, L34, L38, L40, L46, L47, L53, L60, L66, L73, L87, L88, L89, L91, L92, L96, L97, L100.
		Medium	47	L1, L2, L4, L5, L6, L8, L9, L12, L19, L22, L24, L25, L26, L27, L28, L30, L41, L42, L44, L45, L48, L49, L51, L55, L56, L57, L58, L59, L61, L63, L65, L67, L68, L69, L71, L72, L74, L75, L77, L81, L82, L85, L86, L90, L93, L95, L99.
		Narrow	32	L3, L10, L11, L13, L14, L15, L16, L17, L18, L20, L23, L31, L32, L35, L36, L37, L39, L43, L50, L52, L54, L62, L64, L70, L76, L76, L78, L79, L80, L83, L84, L94, L98.
7.	Leaf shape	Oval	32	L1, L2, L4, L8, L22, L23, L29, L30, L33, L34, L35, L38, L40, L41, L46, L47, L53, L57, L59, L61, L65, L66, L73, L74, L87, L88, L89, L92, L96, L97, L98, L100.
		Acute	68	L3, L5, L6, L7, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L21, L24, L25, L26, L27, L28, L31, L32, L36, L37, L39, L42, L43, L44, L45, L48, L49, L50, L51, L52, L54, L55, L56, L58, L60, L62, L63, L64, L67, L68, L69, L70, L71, L72, L75, L76, L77, L78, L79, L80, L81, L82, L83, L84, L85, L86, L90, L91, L93, L94, L95, L99.

Contd.

8.	Leaf colour	Green	38	L1, L4, L5, L6, L7, L12, L13, L16, L19, L21, L22, L24, L25, L26, L27, L28, L38, L42, L44, L48, L51, L54, L56, L57, L58, L59, L60, L62, L67, L77, L80, L81, L83, L84, L90, L93, L95, L99.
		Light green	43	L2, L15, L20, L23, L29, L32, L33, L34, L35, L36, L37, L39, L40, L46, L47, L49, L50, L52, L61, L63, L64, L65, L66, L69, L70, L71, L73, L74, L75, L76, L78, L82, L85, L86, L87, L88, L89, L91, L92, L96, L97, L98, L100.
		Dark green	19	L3, L8, L9, L10, L11, L12, L17, L18, L30, L31, L41, L43, L45, L53, L55, L68, L72, L79, L94.
9.	Stipule size	Long	17	L2, L6, L21, L26, L29, L46, L47, L53, L60, L61, L63, L65, L66, L74, L86, L93, L99.
		Short	83	L1, L3, L4, L5, L7, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L22, L23, L24, L25, L27, L28, L30, L31, L32, L33, L34, L35, L36, L37, L38, L39, L40, L41, L42, L43, L44, L45, L48, L49, L50, L51, L52, L54, L55, L56, L57, L58, L59, L62, L64, L67, L68, L69, L70, L71, L72, L73, L75, L76, L77, L78, L79, L80, L81, L82, L83, L84, L85, L86, L87, L88, L89, L90, L91, L92, L94, L95, L96, L97, L98, L100.
10.	Peduncle length	Long	41	L2, L6, L8, L10, L11, L13, L15, L16, L19, L24, L25, L29, L30, L31, L32, L37, L39, L40, L41, L42, L45, L54, L58, L59, L61, L63, L65, L66, L71, L72, L73, L74, L80, L82, L85, L88, L89, L91, L92, L98, L100.
		Short	59	L1, L3, L4, L5, L7, L9, L12, L14, L17, L18, L20, L21, L22, L23, L26, L27, L28, L33, L34, L35, L36, L38, L43, L44, L46, L47, L48, L49, L50, L51, L52, L53, L55, L56, L57, L60, L62, L64, L67, L68, L69, L70, L75, L76, L77, L78, L79, L81, L83, L84, L85, L87, L90, L93, L94, L95, L96, L97, L99.
11.	Flower colour	Purple	48	L1, L3, L8, L10, L12, L13, L14, L15, L16, L17, L18, L19, L22, L29, L31, L32, L38, L39, L45, L51, L53, L56, L59, L64, L67, L68, L71, L72, L73, L74, L75, L76, L77, L78, L79, L81, L82, L84, L85, L86, L87, L88, L90, L91, L93, L94, L95, L99.
		Light purple	26	L2, L5, L6, L7, L9, L11, L21, L23, L24, L25, L26, L27, L28, L30, L42, L44, L57, L63, L65, L69, L70, L80, L83, L89, L92, L98.
		Dark purple	5	L4, L58, L96, L97, L100.
		White	21	L20, L33, L34, L35, L36, L37, L40, L41, L43, L46, L47, L48, L49, L50, L52, L54, L55, L60, L61, L62, L66.

Contd.

12.	Testa colour	Brown	37	L2, L3, L4, L11, L18, L20, L22, L23, L25, L26, L31, L37, L38, L39, L41, L45, L50, L51, L54, L59, L61, L62, L63, L64, L65, L67, L68, L71, L73, L74, L75, L79, L80, L81, L90, L94, L95.
		Buff	13	L69, L77, L86, L87, L88, L89, L90, L91, L92, L96, L97, L98, L100.
		Black	5	L5, L12, L15, L49, L58.
		Green	17	L6, L7, L10, L14, L16, L17, L21, L27, L42, L48, L55, L56, L60, L78, L83, L84.
		Gray	15	L8, L9, L13, L19, L24, L28, L29, L30, L32, L57, L70, L76, L82, L93, L99.
		Tan	12	L33, L34, L35, L36, L40, L43, L46, L47, L52, L53, L66, L72.
		Yellow	1	L44.
13.	Testa pattern	Mottled	69	L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L21, L22, L23, L24, L25, L26, L27, L28, L29, L30, L31, L32, L33, L38, L39, L40, L42, L43, L44, L45, L51, L53, L55, L56, L57, L58, L59, L60, L62, L63, L64, L67, L68, L70, L71, L78, L80, L81, L82, L84, L85, L86, L88, L90, L92, L93, L94, L95, L99.
		Non-mottled	31	L34, L35, L36, L37, L41, L46, L47, L48, L49, L50, L52, L54, L61, L65, L66, L69, L72, L73, L74, L75, L76, L77, L79, L83, L87, L89, L91, L96, L97, L98, L100.
14.	Seed shape	Spherical	58	L1, L3, L4, L5, L8, L9, L10, L11, L12, L13, L14, L15, L16, L18, L19, L20, L22, L23, L24, L26, L28, L29, L31, L32, L41, L45, L51, L56, L57, L58, L59, L62, L64, L67, L68, L70, L71, L74, L75, L76, L77, L78, L79, L80, L81, L82, L83, L84, L85, L88, L89, L90, L91, L92, L93, L94, L95, L96, L99.
		Flat	40	L2, L6, L7, L17, L21, L25, L27, L30, L33, L34, L35, L36, L37, L38, L39, L40, L42, L43, L44, L46, L47, L48, L49, L50, L54, L55, L58, L60, L61, L63, L65, L66, L69, L72, L73, L86, L87, L97, L98, L100.
		Globe	1	L53.

Contd.

		Oval	1	L52.
15.	Cotyledon colour	Orange	53	L1, L3, L4, L5, L6, L7, L8, L9, L10, L12, L13, L15, L18, L19, L22, L23, L25, L29, L31, L32, L35, L42, L44, L46, L50, L51, L52, L53, L57, L58, L59, L61, L63, L64, L67, L68, L69, L70, L71, L72, L77, L81, L82, L84, L86, L87, L88, L89, L90, L91, L92, L93, L95.
		Yellow	38	L2, L20, L21, L26, L27, L33, L34, L37, L38, L39, L40, L41, L43, L45, L47, L48, L49, L54, L55, L56, L60, L62, L65, L66, L73, L74, L75, L76, L78, L79, L83, L85, L94, L96, L97, L98, L99, L100.
		Green	6	L8, L11, L24, L28, L30, L80.
		Light green	2	L16, L17
		Brown	1	L14.

11. Flower colour

Four types of flower colours were observed viz. purple, light purple, dark purple and white. Forty eight genotypes were purple flowered and twenty six genotypes were light purple flowered. However five genotypes were dark purple flowered and twenty one genotypes were white flowered

12. Testa colour

Observed seven types of testa colours in lentil in the materials under study viz., brown, buff, black, green gray, tan and yellow. Recorded number of genotypes, respectively, in different categories were thirteen, five, seventeen, fifteen, twelve and one out of 100 lines and varieties.

13. Testa pattern

Found only two types of testa pattern in lentil in the present study viz. mottled and non-mottled. Sixty-nine genotypes were mottled and thirty-one genotypes were non-mottled out of 100 lines and varieties, investigated in the present study.

14. Seed shape

Four types of seed shape in lentil like spherical, flat, globe and oval were observed. Fifty eight genotypes were having spherical seeds, forty genotype were flat only one genotypes each was globe and oval.

15. Cotyledon colour

Found four different types of cotyledon colours in lentil viz., orange, yellow green and brown. Fifty three genotypes were orange thirty eight genotypes was yellow, eight genotypes was green and only one genotypes was brown out of 100 lines and varieties evaluated.

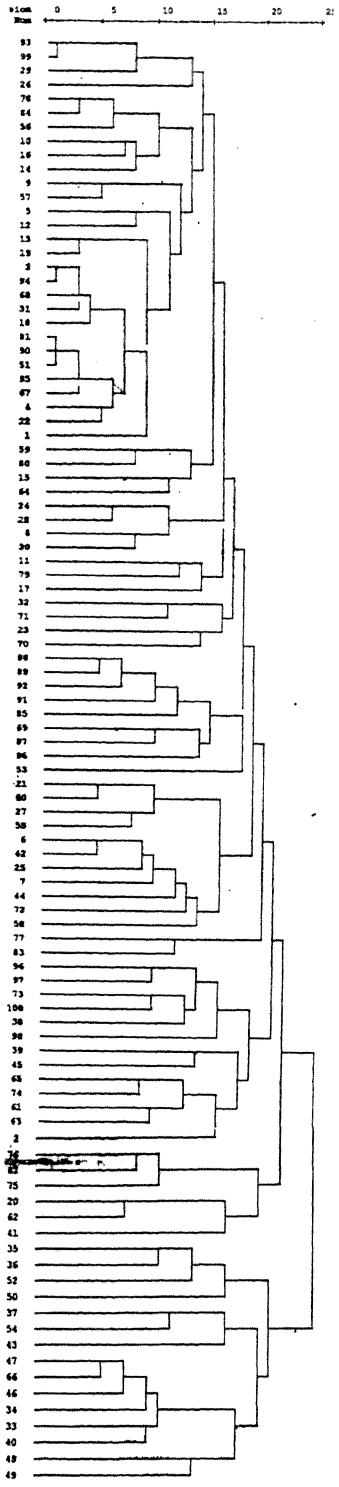
4.4.1 Clustering of genotypes

On the basis of morphological analysis dendrogram (fig.6) 100 germplasm lines and varieties under study have been grouped in to ten cluster (Table 4.3). The Cluster Ist had the maximum lines (29), followed by the clusters X (15), VIII (13), VI(11), V(9), III (7), IX(6). On the other hand, cluster II and IV had 4 lines each and VII clusters had 2 genotypes.

Table 4.3 Distribution of 100 germplasm lines of lentil in different cluster based on morphological analysis.

Cluster	Number of germplasm lines	Names (symbol) of germplasm lines
I	29	L1, L3, L4, L5, L9, L10, L12, L13, L14, L16, L18, L19, L22, L26, L29, L31, L51, L57, L56, L67, L68, L78, L81, L84, L90, L93, L94, L95, L99.
II	4	L15, L59, L64, L80.
III	7	L8, L11, L17 L24, L28, L30, L79.
IV	4	L23, L32, L70, L71.
V	9	L53, L69, L85, L86, L87, L88, L89, L91, L92.
VI	11	L6, L7, L21, L25, L27, L42, L44, L55, L58, L60, L72.
VII	2	L77, L83.
VIII	13	L2, L38, L39, L45, L61, L63, L65, L73, L74, L96, L97, L98, L100.
IX	6	L20, L41, L62, L75, L76, L82.
X	15	L33, L34, L35, L36, L37, L40, L43, L46, L47, L48, L49, L50, L52, L54, L66.

Fig. 6· Dendrogram of 100 germplasm of lentil based on qualitative analysis



4.2 Quantitative traits

4.2.1 Analysis of variance

The data recorded on twelve quantitative characters were analysed for augmented randomized complete block design and the mean sum of squares (MSS) due to different sources of variation are given in (Table 4.4).

A perusal of the in (Table 4.4) suggests that the variation due to replications was not significant for all the characters, excepting days to maturity where it was significant at 5% level of probability only. This indicates suggested presence of uniformly in the experimental area.

On the other hand, this statistical parameter was found highly significant for all the twelve characters for all the germplasm lines, including varieties and checks, signifying the presence of immense genetic variability among them.

The total variance due to all 100 germplasm lines was also partitioned in order to assess the magnitude of variability, each among un-replicated germplasm lines. The MSS due to each un-replicated germplasm line and replicated checks were found highly significant for all the characters under study. This suggested that genetic variability existed among the component lines of each of these two sets of populations. The differences between these two sets of population i.e. 97 germplasm lines and 3 checks, were also found highly significant suggesting that these two groups are genetically distinct.

Table 4.4 ANOVA of 12 characters of 100 germplasm lines of lentil in augmented block design.

Source of variation	d.f.	M.S.S											
		1	2	3	4	5	6	7	8	9	10	11	12
Block	2	1153.85	143.09	296.41	34.76	21.21	49.81	1117.97	31.61	37.98	53.46	319.59	34.60
Treatment	99	123.63**	45.89**	88.69**	8.25**	6.08**	12.24**	990.76**	7.05**	9.25**	13.32**	105.50**	35.71**
Error	4	2.66	0.11	0.94	0.77	0.41	0.32	5.12	0.13	0.33	0.33	0.14	0.95

** Significant at 1% level of significance.

1=Days to flowering, 2 =Days to maturity, 3= Plant height, 4=No. of pri. br., 5= No. of sec. br, 6=No.of flowers per peduncle, 7=Pod per plant, 8=Seed per pod, 9=100-seed wt., 10=Grain yield, 11=biomass score, 12= Harvest index

4.2.2 Mean, range and variation

a. Mean and range

The data recorded on 12 metric traits in 3 checks (Sehore74-3, L4076, Precoz) replicated 3 times and 97 un-replicated germplasm lines grown in augmented design have been presented in (Table 4.5) respectively. As per the procedure of augmented randomized complete block design the mean data from 100 germplasm lines were adjusted and the values recorded over 3 checks of replication were averaged. The adjusted mean values of 100 germplasm lines have been given in (Table 4.6). These values have been used in subsequent analyses and describing the results on mean and range for each of the twelve characters as follows:

1. Days to flowering

The mean number of days to flowering has been found 77.84 in germplasm lines. However, the range for this developmental character has been found wider (49.50-101.50 days) among the germplasm lines.

2. Days to maturity

The average maturity duration in the germplasm lines was 125.0 days. On the other hand, the range for this period was wider among the germplasm lines (109.50-141.00 days).

3. Plant height

The mean plant height has been found 37.65cm for germplasm lines. The range for plant height was found to be 14.50-62.00 cm among the germplasm lines.

Table 4.5 Mean performance of 100 germplasm lines and varieties of lentil.

S.No.	Accession Symbol	Days to flowering	Days to maturity	Plant height (cm)	No. of pri. branches	No. of sec. branches	No. of flower/ peduncle	Pods/plant	No. of seed/pod	100 seed weight (g)	Grain yield (g)	Biomass score (g)	Harvest Index (%)
1.	L1	68.00	131.0	42.00	1.50	12.30	1.95	79.40	1.67	2.60	5.43	18.69	29.05
2.	L2	73.00	123.0	40.00	2.10	8.50	2.05	113.00	1.53	3.58	5.43	18.61	29.18
3.	L3	74.50	125.5	47.00	1.70	8.70	1.90	130.60	1.71	1.79	6.94	19.80	35.05
4.	L4	82.50	128.5	48.00	2.10	9.70	2.10	108.90	1.57	2.99	6.38	38.20	16.70
5.	L5	83.50	129.0	50.50	2.35	10.50	1.85	130.70	1.70	2.27	5.65	25.39	22.25
6.	L6	82.50	127.5	46.00	2.25	10.90	3.15	159.10	1.32	2.75	4.71	16.51	28.52
7.	L7	79.00	119.0	28.00	1.95	12.70	1.95	155.50	1.52	2.35	5.73	20.40	28.09
8.	L8	95.00	135.0	52.50	1.65	11.80	2.05	112.85	1.36	2.35	6.80	21.00	32.38
9.	L9	87.00	134.5	52.00	2.55	10.90	3.00	127.20	1.64	1.63	4.65	15.45	30.10
10.	L10	93.00	134.5	62.00	1.75	7.10	2.00	137.00	1.84	2.15	5.65	29.15	19.38
11.	L11	91.50	132.5	52.50	2.25	10.50	2.00	158.60	1.91	2.10	6.61	25.20	26.23
12.	L12	101.5	140.5	47.50	2.15	11.90	3.00	145.40	1.50	1.81	6.99	23.55	29.86
13.	L13	87.00	133.0	41.50	2.05	11.90	1.95	137.40	1.67	2.05	5.66	22.85	24.84
14.	L14	85.00	137.0	41.50	1.95	13.50	2.05	122.80	1.81	2.61	6.74	19.95	33.78
15.	L15	94.50	141.0	49.00	2.25	10.50	2.90	96.60	1.40	1.55	3.82	13.25	28.83
16.	L16	69.50	133.0	59.00	1.55	7.30	2.10	124.20	1.52	2.20	3.83	22.90	16.72
17.	L17	77.00	127.0	50.50	2.45	9.30	3.00	96.60	1.48	2.45	5.39	18.70	28.83
18.	L18	89.00	135.0	48.50	1.95	10.90	3.00	104.00	1.72	1.56	5.47	18.55	29.49
19.	L19	89.00	129.5	45.50	2.45	9.30	1.90	131.20	1.84	1.28	6.94	19.85	34.96
20.	L20	67.00	114.5	30.50	1.80	15.20	2.10	134.00	1.60	2.85	6.38	38.20	16.70
21.	L21	70.50	119.0	32.50	2.05	15.80	1.90	136.60	1.55	3.45	5.61	25.40	22.08
22.	L22	87.50	122.0	48.50	1.90	8.90	2.10	150.60	1.57	1.58	4.75	16.45	28.88
23.	L23	101.0	129.5	14.50	2.30	17.10	1.95	151.00	1.62	1.24	5.73	20.45	28.02
24.	L24	94.00	123.5	48.50	2.10	14.70	3.05	172.30	1.66	1.65	6.80	21.00	32.38
25.	L25	94.50	127.0	25.50	2.10	11.90	1.90	128.20	1.55	1.10	4.70	15.50	30.32

Contd.

26.	L26	100.5	131.0	23.50	2.30	16.20	3.10	136.00	1.33	2.70	5.60	29.10	19.24
27.	L27	80.50	121.0	23.00	2.10	14.40	1.95	158.60	1.31	1.74	6.61	25.40	26.02
28.	L28	79.50	120.0	45.00	2.10	7.70	2.05	137.00	1.61	2.38	6.94	21.70	31.98
29.	L29	76.00	121.5	34.00	2.30	8.10	1.90	121.80	1.51	3.77	5.76	19.95	28.88
30.	L30	81.00	118.5	49.00	2.10	8.90	2.10	98.60	1.21	2.90	4.43	13.25	33.47
31.	L31	77.00	116.0	41.50	1.90	11.10	1.95	96.60	1.24	2.16	3.82	13.20	28.94
32.	L32	64.00	111.0	38.50	2.10	8.50	2.05	122.20	1.72	3.22	3.83	22.90	16.72
33.	L33	90.50	131.0	26.00	2.90	10.30	2.00	96.60	1.24	3.06	5.39	18.70	28.83
34.	L34	99.50	131.5	24.50	2.20	10.10	3.00	104.00	1.28	2.11	5.47	18.55	29.49
35.	L35	95.50	131.0	29.00	2.20	9.70	4.00	131.15	1.16	3.85	6.94	19.85	34.96
36.	L36	89.50	121.0	26.00	2.55	7.80	4.00	137.15	1.36	2.81	6.38	38.20	16.70
37.	L37	61.00	109.0	27.00	1.70	14.00	2.00	139.00	1.24	3.81	5.61	25.40	22.08
38.	L38	96.00	134.0	50.00	2.35	8.70	3.00	148.60	1.05	3.79	4.75	15.90	29.88
39.	L39	61.50	111.0	27.00	2.05	11.90	2.00	137.00	1.47	4.13	5.73	20.45	28.02
40.	L40	73.50	120.0	46.00	1.55	9.00	2.90	152.00	1.45	4.41	6.80	21.00	32.38
41.	L41	67.00	124.0	45.50	2.05	11.50	2.10	128.00	1.55	2.53	4.70	15.50	30.32
42.	L42	66.00	121.0	34.50	1.15	8.30	2.95	136.00	1.29	3.84	5.60	29.10	19.24
43.	L43	62.50	113.0	18.50	2.05	10.10	2.05	141.00	1.43	3.00	6.61	25.10	26.02
44.	L44	72.50	121.0	32.50	2.70	13.30	1.95	138.40	1.49	3.82	6.94	21.70	31.98
45.	L45	91.00	139.5	20.50	2.10	6.30	2.05	121.80	1.27	3.66	6.74	19.95	33.78
46.	L46	94.00	134.5	26.00	2.75	12.10	2.95	100.90	1.33	3.12	3.77	13.25	28.45
47.	L47	59.00	109.5	26.00	2.45	8.70	3.05	122.20	1.12	5.58	3.88	22.85	16.98
48.	L48	61.00	110.5	27.50	1.70	8.70	2.00	98.60	1.40	4.20	5.39	18.75	28.74
49.	L49	62.00	120.5	36.50	2.25	6.10	3.00	105.50	1.24	2.81	5.47	18.55	29.49
50.	L50	75.00	123.5	25.50	2.55	14.10	3.00	135.70	1.24	2.87	6.94	19.85	34.96
51.	L51	68.50	118.0	24.50	1.85	11.60	2.00	137.30	1.63	1.87	6.38	38.20	16.70
52.	L52	72.50	135.0	27.00	1.95	10.20	1.90	141.25	1.57	2.64	5.66	25.40	22.28
53.	L53	81.50	129.0	28.50	1.85	11.10	3.10	149.60	1.25	2.02	4.70	16.50	28.48
54.	L54	63.50	121.0	33.50	2.55	14.00	1.90	152.20	1.45	4.52	5.73	20.40	28.09

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55.	L55	87.00	126.0	38.50	3.25	11.20	2.10	170.00	1.56	2.87	6.80	21.00	32.38
56.	L56	91.00	128.5	44.50	2.55	8.40	1.95	120.70	1.52	1.81	4.70	15.50	30.32
57.	L57	87.00	130.5	44.50	2.85	5.30	2.05	131.00	1.63	2.71	5.60	29.10	19.24
58.	L58	94.00	131.0	40.50	2.45	10.90	1.95	141.00	1.49	3.49	6.61	25.40	26.02
59.	L59	92.00	127.0	50.50	2.05	5.50	3.05	130.00	1.35	2.98	6.99	21.70	32.21
60.	L60	61.00	121.5	39.50	2.10	9.50	1.90	99.10	1.77	4.24	3.56	13.20	26.97
61.	L61	70.50	120.5	45.50	2.10	6.50	2.10	110.70	1.15	4.47	3.84	22.89	16.77
62.	L62	86.50	121.5	31.00	1.95	9.50	2.95	91.10	1.37	4.77	5.42	18.71	28.97
63.	L63	60.50	128.5	29.00	3.05	7.50	2.05	128.20	1.20	3.79	5.44	18.58	29.28
64.	L64	85.50	121.0	39.00	1.90	9.50	2.00	129.00	1.28	4.69	6.92	19.82	34.92
65.	L65	61.00	126.0	39.00	2.10	6.50	2.00	130.50	1.48	4.69	6.40	38.18	16.76
66.	L66	65.00	121.5	29.50	2.95	12.50	3.00	137.10	1.60	3.40	5.66	25.42	22.26
67.	L67	62.00	126.5	45.50	2.95	8.10	2.00	141.60	1.00	7.40	11.33	58.70	19.30
68.	L68	73.00	127.0	44.50	2.55	8.70	1.90	139.40	2.00	5.69	8.39	30.90	27.15
69.	L69	45.50	116.0	31.00	2.85	5.90	3.10	62.20	2.00	3.59	5.44	18.70	29.09
70.	L70	60.50	119.0	22.50	3.35	12.50	1.90	112.00	1.80	3.78	5.42	18.60	29.14
71.	L71	64.00	121.0	35.50	3.65	8.10	2.10	130.70	1.40	5.72	6.94	19.80	35.05
72.	L72	66.00	128.0	33.50	2.95	12.30	1.90	155.70	1.80	4.06	7.95	53.00	15.00
73.	L73	77.00	133.0	48.50	3.65	13.40	2.10	173.20	1.60	4.67	13.49	61.00	22.11
74.	L74	81.00	135.0	46.00	3.35	10.20	2.00	108.40	1.55	3.97	6.38	38.20	16.70
75.	L75	73.00	131.0	32.00	4.85	8.70	2.00	130.50	1.45	4.64	5.66	28.40	19.93
76.	L76	76.00	131.5	41.00	3.55	14.00	2.00	146.40	1.35	4.57	7.40	43.50	17.01
77.	L77	70.50	125.5	38.00	2.65	9.40	3.00	157.70	2.05	3.74	4.70	16.50	28.48
78.	L78	64.50	122.0	35.50	2.90	14.20	2.00	157.00	1.55	2.70	5.72	20.39	28.03
79.	L79	79.00	126.0	37.50	2.90	13.50	2.00	210.50	1.65	3.48	8.83	33.42	26.41
80.	L80	65.00	125.0	39.00	2.85	14.60	2.00	214.10	1.95	3.15	11.15	33.85	32.94
81.	L81	73.00	121.0	43.00	3.15	10.20	2.00	172.20	1.65	4.71	6.85	21.05	32.54
82.	L82	82.00	125.0	36.00	2.90	11.20	1.85	128.00	1.80	3.75	4.68	15.48	30.21
83.	L83	61.00	119.0	38.50	2.70	11.00	2.15	126.40	2.40	3.71	7.22	19.12	37.78
84.	L84	75.00	123.0	25.50	2.70	13.80	1.90	137.00	1.60	4.69	5.60	29.10	19.24
85.	L85	87.00	127.0	31.00	4.30	15.00	2.10	187.80	1.80	4.71	6.80	29.10	23.37

Contd.

86.	L86	81.00	121.0	41.00	2.75	12.60	1.95	210.00	1.20	3.26	7.63	32.30	23.62
87.	L87	81.50	126.0	33.50	2.25	6.50	2.05	201.00	2.00	5.61	8.27	43.00	19.23
88.	L88	66.50	121.0	33.50	1.95	11.50	1.95	174.00	1.75	3.71	12.08	57.70	20.94
89.	L89	72.00	123.0	29.50	2.45	7.10	2.05	158.60	1.45	5.21	6.61	25.00	26.44
90.	L90	82.00	124.0	30.50	2.20	9.10	1.90	145.40	1.35	3.26	6.94	25.35	27.38
91.	L91	86.50	126.0	42.00	3.05	7.30	2.10	137.40	1.85	4.68	5.76	21.75	26.47
92.	L92	76.50	118.0	33.00	2.10	10.30	1.95	122.80	1.40	5.41	6.69	20.01	33.47
93.	L93	62.50	120.0	41.50	2.30	6.90	2.10	69.00	1.80	1.43	2.20	6.89	31.98
94.	L94	78.50	119.0	51.50	1.95	7.70	2.00	98.60	1.75	2.26	3.82	13.20	28.97
95.	L95	69.00	125.0	29.00	2.25	9.60	2.00	44.60	1.05	1.32	2.06	7.79	26.44
96.	L96	88.00	118.0	44.00	2.70	12.40	1.85	123.20	1.90	1.43	3.84	22.91	16.76
97.	L97	88.50	127.0	42.00	2.70	10.70	3.15	96.60	1.90	3.87	6.08	30.80	19.74
98.	L98	89.50	126.0	44.00	2.90	12.30	1.95	54.40	1.75	1.22	2.41	20.60	11.70
99.	L99	69.50	121.0	43.00	2.50	7.90	2.10	63.40	2.05	1.10	2.67	8.88	30.06
100.	L100	74.50	121.0	32.00	2.30	7.30	2.00	179.20	1.80	3.81	9.79	55.82	17.54
Mean of the lines		**77.84**	**125.0**	**37.65**	**2.39**	**10.41**	**2.27**	**131.57**	**1.54**	**3.21**	**6.00**	**24.24**	**26.32**
		49.50	**109.5**	**14.50**	**1.15**	**5.50**	**1.85**	**44.60**	**1.00**	**1.10**	**2.06**	**6.89**	**11.70**
Range among		-	-	-	-	-	-	-	-	-	-	-	-
the lines		**101.5**	**141.0**	**62.00**	**4.85**	**17.10**	**4.00**	**214.10**	**2.40**	**7.40**	**13.49**	**61.00**	**37.78**

Table 4.6 Adjusted mean values of 12 characters of 100 germplasm lines of lentil.

S.No.	Accession symbol	Days to flowering	Days to Maturity	Plant height (cm)	No. of pri. branches	No. of sec. branches	No. of flower\ peduncle	Pods/plant	No. of seed/ pod	100 seed weight (g)	Grain yield (g)	Biomass score (g)	Harvest index (%)
1.	L1	67.66	131.00	42.00	1.56	12.13	1.96	80.26	1.68	2.65	5.61	18.79	29.85
2.	L2	87.00	134.33	51.66	2.53	10.60	3.00	126.46	1.62	1.78	4.64	15.48	29.96
3.	L3	64.33	121.33	29.66	2.93	12.00	3.00	135.60	1.70	3.33	5.80	25.11	23.14
4.	L4	71.00	120.55	36.44	1.94	7.51	1.98	111.60	1.49	3.54	5.50	18.53	29.56
5.	L5	75.00	124.55	47.44	1.74	8.71	1.98	130.24	1.77	1.80	7.02	19.73	35.47
6.	L6	80.00	126.55	44.44	1.94	8.71	1.98	107.44	1.53	3.00	6.46	38.13	17.12
7.	L7	83.00	128.55	49.44	2.34	10.51	1.98	130.44	1.71	2.29	5.74	25.33	22.70
8.	L8	81.00	125.55	45.44	2.14	9.91	2.98	157.64	1.33	2.75	4.78	16.43	28.90
9.	L9	79.00	118.55	27.44	1.94	12.71	1.98	155.04	1.53	2.41	5.81	20.33	28.51
10.	L10	93.00	132.55	49.44	1.54	10.71	1.98	111.34	1.37	2.31	6.88	20.93	32.80
11.	L11	90.00	131.55	58.44	1.74	6.11	1.98	135.04	1.85	2.11	5.68	29.03	19.66
12.	L12	92.00	131.55	53.44	2.14	10.51	1.98	157.64	1.97	2.12	6.69	24.93	26.86
13.	L13	99.00	138.55	43.44	2.14	10.91	2.98	144.44	1.45	1.81	7.07	25.33	27.94
14.	L14	87.00	132.55	41.44	1.94	11.91	1.98	136.44	1.73	2.11	5.79	21.63	26.73
15.	L15	83.00	134.55	38.44	1.94	12.51	1.98	121.84	1.77	2.57	6.77	19.93	33.87
16.	L16	94.00	140.55	48.44	2.14	10.51	2.98	96.64	1.41	1.61	3.90	13.13	29.36
17.	L17	68.00	130.55	56.44	1.54	6.31	1.98	122.24	1.53	2.16	3.91	22.83	17.14
18.	L18	77.00	126.55	49.44	2.34	9.31	2.98	96.64	1.49	2.51	5.52	18.63	29.51
19.	L19	87.00	132.55	46.44	1.94	9.91	2.98	102.04	1.73	1.52	5.50	18.53	29.56
20.	L20	89.00	128.55	44.44	2.34	9.31	1.98	131.24	1.85	1.29	7.02	19.73	35.47
21.	L21	65.00	112.55	28.44	1.74	15.71	1.98	132.04	1.61	2.86	6.46	38.13	17.12
22.	L22	70.00	118.55	33.44	1.94	14.31	1.98	135.64	1.61	3.46	5.74	25.33	22.70
23.	L23	86.00	119.55	44.44	1.94	8.91	1.98	149.64	1.53	1.59	4.78	16.43	28.90
24.	L24	101.00	128.55	13.44	2.14	16.11	1.98	150.04	1.65	1.25	5.81	20.33	28.51
25.	L25	92.00	121.55	46.44	2.14	14.71	2.98	171.34	1.65	1.66	6.88	20.93	32.80

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26.	L26	95.00	126.55	24.44	1.94	10.91	1.98	127.24	1.61	1.11	4.78	15.43	30.74
27.	L27	98.00	128.55	20.44	2.34	16.71	2.98	135.04	1.29	2.71	5.68	29.03	19.66
28.	L28	80.00	120.55	22.44	1.94	13.11	1.98	157.64	1.37	1.80	6.69	25.33	26.44
29.	L29	78.00	117.55	42.44	2.14	7.71	1.98	136.44	1.57	2.34	7.07	21.63	32.63
30.	L30	76.00	121.55	33.44	2.14	7.11	1.98	121.84	1.57	3.83	5.79	19.93	28.97
31.	L31	79.00	115.55	46.44	2.14	8.91	1.98	96.64	1.17	2.86	4.50	13.13	33.90
32.	L32	77.00	115.55	40.44	1.74	10.11	1.98	96.64	1.25	2.23	3.90	13.13	29.36
33.	L33	62.00	108.55	36.44	2.14	8.51	1.98	120.24	1.73	3.18	3.91	22.83	17.14
34.	L34	90.00	129.55	25.44	2.74	9.31	1.98	96.64	1.25	3.07	5.52	18.63	29.51
35.	L35	98.00	129.55	22.44	2.34	10.11	2.98	102.04	1.29	2.12	5.50	18.53	29.56
36.	L36	95.00	130.55	28.44	1.94	8.71	3.98	131.34	1.17	3.86	7.02	19.73	35.47
37.	L37	89.00	121.22	26.44	2.67	8.64	4.02	136.34	1.41	2.89	6.50	38.15	17.31
38.	L38	62.33	111.22	29.44	1.67	12.84	2.02	139.04	1.29	3.89	5.78	25.35	22.89
39.	L39	95.33	134.22	50.44	2.47	9.04	3.02	148.04	1.10	3.87	4.82	15.90	30.08
40.	L40	63.33	113.22	29.44	2.07	11.24	2.02	138.04	1.57	4.21	5.85	20.35	28.70
41.	L41	72.33	120.22	46.44	1.67	9.44	3.02	151.04	1.45	4.49	6.92	20.95	32.99
42.	L42	68.33	126.22	47.44	2.07	10.84	2.02	128.04	1.65	2.66	4.82	15.45	30.93
43.	L43	65.33	121.22	35.44	1.27	8.64	3.02	136.04	1.29	3.87	5.72	29.05	19.85
44.	L44	65.33	115.22	20.44	2.07	9.44	2.02	142.04	1.53	3.08	6.73	25.35	26.63
45.	L45	70.33	121.22	33.44	2.87	12.64	2.02	137.44	1.49	3.90	7.11	21.65	32.82
46.	L46	92.33	141.22	22.44	2.07	6.64	2.02	122.84	1.37	3.79	6.81	19.95	34.06
47.	L47	93.33	135.22	28.44	2.87	11.44	3.02	99.94	1.33	3.15	3.94	13.15	29.55
48.	L48	60.33	111.22	26.44	2.47	9.04	3.02	122.24	1.17	5.66	3.95	22.85	17.33
49.	L49	60.33	111.22	29.44	1.87	8.04	2.02	98.64	1.45	4.28	5.56	18.65	29.70
50.	L50	84.33	122.22	37.44	2.27	6.44	3.02	106.04	1.29	2.94	5.54	18.55	29.75
51.	L51	73.33	124.22	27.44	2.67	13.44	3.02	133.24	1.29	2.90	7.06	19.75	35.66
52.	L52	69.33	120.22	25.44	1.87	12.44	2.02	139.34	1.73	2.00	6.50	38.15	17.31
53.	L53	72.33	135.22	29.44	2.07	9.04	2.02	142.34	1.57	2.67	5.78	25.35	22.89
54.	L54	83.33	131.22	29.44	1.87	11.44	3.02	149.64	1.35	2.10	4.82	16.45	29.09
55.	L55	62.33	123.22	35.44	2.67	13.24	2.02	152.24	1.45	4.60	5.85	20.35	28.70
56.	L56	88.33	126.22	39.44	3.27	12.04	2.02	171.34	1.61	3.00	6.92	20.95	32.99
57.	L57	90.33	130.22	46.44	2.67	7.24	2.02	120.24	1.57	1.84	4.82	15.45	30.93
58.	L58	89.33	131.22	45.44	2.87	5.64	2.02	132.04	1.73	2.84	5.72	29.05	19.85
59.	L59	92.33	133.22	42.44	2.47	10.24	2.02	140.04	1.49	3.52	6.73	25.35	26.63
60.	L60	93.33	127.22	51.44	2.07	5.84	3.02	130.04	1.45	3.06	7.11	21.65	32.82

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61.	L61	60.33	124.22	41.44	2.27	8.84	2.02	99.14	1.77	4.32	3.94	13.15	29.55
62.	L62	71.33	120.22	46.44	2.07	6.84	2.02	111.24	1.25	4.60	3.95	22.85	17.33
63.	L63	86.33	124.22	33.44	2.07	7.84	3.02	90.64	1.37	4.80	5.56	18.65	29.70
64.	L64	61.33	128.22	29.44	3.07	7.84	2.02	128.24	1.25	3.87	5.54	18.55	29.75
65.	L65	85.33	123.22	41.44	2.07	8.84	2.02	129.04	1.33	4.77	7.06	19.75	35.66
66.	L66	62.33	128.22	39.44	2.07	6.84	2.02	131.04	1.53	4.77	6.50	38.15	17.31
67.	L67	64.33	126.22	46.44	2.67	8.44	2.02	141.64	1.05	7.48	11.45	58.65	19.91
68.	L68	71.33	129.22	46.44	2.67	8.04	2.02	139.44	2.05	5.77	8.51	30.85	27.76
69.	L69	50.33	116.22	31.44	2.87	6.24	3.02	62.24	2.05	3.67	5.56	18.65	29.70
70.	L70	60.66	120.22	23.11	3.37	12.64	1.98	112.91	1.73	3.68	5.20	18.71	20.10
71.	L71	65.66	120.22	35.11	3.57	9.24	1.98	132.11	1.33	5.62	6.72	19.91	34.01
72.	L72	65.66	129.22	34.11	2.97	12.44	1.98	156.11	1.73	3.96	7.73	53.11	13.96
73.	L73	78.66	132.22	48.11	3.57	15.04	1.98	174.11	1.53	4.57	13.27	61.11	21.07
74.	L74	80.66	136.22	47.11	3.37	9.84	1.98	109.31	1.53	3.87	6.16	38.31	15.66
75.	L75	74.66	130.22	31.11	4.77	10.84	1.98	131.91	1.33	4.59	5.44	28.51	18.89
76.	L76	75.66	133.22	42.11	3.57	13.64	1.98	147.31	1.33	4.42	7.18	43.61	15.97
77.	L77	71.66	124.22	37.11	2.57	11.04	2.98	159.51	1.93	3.69	4.48	16.61	27.44
78.	L78	64.66	124.22	36.11	2.97	13.84	1.98	156.91	1.53	2.55	5.51	20.51	27.05
79.	L79	80.66	124.22	37.11	2.77	15.64	1.98	211.91	1.53	3.43	8.59	33.51	25.34
80.	L80	64.66	126.22	40.11	2.97	14.24	1.98	214.51	1.93	3.00	10.98	34.01	32.00
81.	L81	74.66	126.22	42.11	2.97	11.84	1.98	173.11	1.53	4.61	6.58	21.11	31.34
82.	L82	81.66	120.22	37.11	2.97	10.84	1.98	128.91	1.73	3.65	4.48	15.61	29.28
83.	L83	62.66	126.22	39.11	2.57	12.64	1.98	128.91	2.33	3.61	6.98	19.21	36.66
84.	L84	74.66	118.22	25.11	2.77	13.44	1.98	136.91	1.53	4.59	5.38	29.21	18.20
85.	L85	88.66	124.22	32.11	4.17	16.64	1.98	189.71	1.73	4.61	6.58	29.21	22.33
86.	L86	80.66	126.22	40.11	2.77	12.24	1.98	209.91	1.13	3.16	7.41	32.41	22.58
87.	L87	83.66	122.22	34.11	2.17	7.64	1.98	202.91	1.93	5.51	8.05	43.11	18.19
88.	L88	65.66	127.22	33.11	1.97	11.64	1.98	175.31	1.73	3.61	11.86	57.81	19.90
89.	L89	74.66	122.22	30.11	2.37	8.24	1.98	159.51	1.33	5.11	6.39	25.11	25.40
90.	L90	80.66	125.22	30.11	2.17	9.24	1.98	146.31	1.33	3.16	6.77	25.51	26.48

Contd.

91.	L91	87.66	125.22	44.11	2.97	8.44	1.98	138.31	1.73	4.63	5.49	21.81	25.27
92.	L92	76.66	119.22	31.11	2.17	10.44	1.98	123.71	1.33	5.26	6.47	20.11	32.41
93.	L93	65.66	119.22	43.11	2.17	8.04	1.98	70.91	1.73	1.38	1.99	7.01	30.99
94.	L94	76.66	120.22	50.11	1.97	7.84	1.98	98.51	1.73	2.11	3.60	13.31	27.90
95.	L95	70.66	124.22	30.11	2.17	11.24	1.98	45.51	0.93	1.22	1.85	7.91	25.50
96.	L96	87.66	119.22	43.11	2.77	12.04	1.98	124.11	1.93	1.33	3.61	23.01	15.68
97.	L97	89.66	126.22	44.11	2.57	11.84	2.98	98.51	1.73	3.77	5.86	30.91	18.70
98.	L98	89.66	129.22	42.11	2.97	12.44	1.98	54.31	1.73	1.12	2.19	20.71	10.66
99.	L99	70.66	120.22	44.11	2.37	9.04	1.98	65.31	1.93	1.00	2.47	9.01	29.18
100.	L100	74.66	120.22	31.11	2.37	7.44	1.98	179.11	1.73	3.71	9.55	55.91	16.47
Mean of the lines		**77.84**	**125.0**	**37.65**	**2.39**	**10.41**	**2.27**	**131.57**	**1.54**	**3.21**	**6.00**	**24.24**	**26.32**
		49.50	**109.5**	**14.50**	**1.15**	**5.50**	**1.85**	**44.60**	**1.00**	**1.10**	**2.06**	**6.89**	**11.70**
Range among		-	-	-	-	-	-	-	-	-	-	-	-
the lines		**101.5**	**141.0**	**62.00**	**4.85**	**17.10**	**4.00**	**214.10**	**2.40**	**7.40**	**13.49**	**61.00**	**37.78**

4. Number of primary branches per plant

The average number of primary branches per plant was 2.39 in the germplasm lines. However, the range for this trait among the germplasm lines was found wider (1.15-4.85).

5. Number of secondary branches per plant

The average number of secondary branches per plant was found to be 10.41 in the germplasm lines. However, the range for this trait among the germplasm lines was 5.50-17.10.

6. Number of flower per peduncle

The average number of flower per peduncle was found fewer (2.27) in germplasm lines. However, the range for this trait among the germplasm lines varied from 1.85 to 4.0.

7. Number of pods per plant

The average number of pods per plant was found to be 131.57 in the germplasm lines. However the range or this trait among the germplasm lines was found to be extremely wider (44.60-214.10).

8. Number of seeds/ pod

The average number of seeds per pod was found to be 1.54 in the germplasm lines. However, the range for this trait among the germplasm lines was wider (1.00-7.40 g).

9. 100-seed weight(g)

The observed mean seed weight of germplasm lines, was 3.21 g. In the germplasm lines, a wide range in seed weight (1.10-7.40g) was also noticed.

10. Grain yield

The average grain yield in case of germplasm lines was 6.00g. In respect of grain yield per plant, the range was extremely wide germplasm lines (2.06-13.49g).

11. Biomass score

The average biomass score of germplasm lines was 24.24g. An extremely wide range in biomass score (6.89-61.00g) was observed.

12. Harvest index

The mean of harvest index of germplasm lines was 26.32%. In the germplasm lines an extremely wide range in harvest index (11.7-37.78%) was noticed.

b Variability

The variance at genotypic and phenotypic levels in respect of 12 characters has been estimated through mean sum of squares (MSS) analysis of variance of the experiment. In order to compare different characters in respect of this parameter the coefficient of variability at the above two levels have been worked out. The estimates of this parameter wise result are presented below:

Table 4.7 Genotypic, phenotypic variability and their coefficient in respect of 12 characters of 100 germplasm lines of lentil.

S.No.	Character	Mean	Variability		Coefficient of variability	
			Genotypic	Phenotypic	Genotypic	Phenotypic
1.	Days to flowering	77. 84	40.32	42.98	15.27	15.40
2.	Days to maturity	125.00	15.26	15.37	5.40	5.52
3.	Plant height (cm)	37.65	29.24	30.19	25.34	25.69
4.	No. of pri. branches	2.39	2.49	3.26	23.90	24.36
5.	No. of sec. branches	10.41	1.89	2.30	24.47	26.19
6.	No. of flower/peduncle	2.27	3.97	4.29	21.89	22.35
7.	Pods/plant	131.57	328.54	333.67	23.57	23.59
8.	No. of seed/pod	1.54	2.30	2.43	20.49	20.86
9.	100 seed wt. (g)	3.21	2.97	3.30	29.04	29.07
10.	Grain yield (g)	6.00	4.32	4.66	23.73	23.75
11.	Biomass score (g)	24.24	35.11	35.26	23.22	23.24
12.	Harvest index (%)	26.32	11.58	12.54	22.65	22.75

1. Days to flowering

The coefficient of variability for days to flowering at genotypic (15.27%) and phenotypic (15.40%) levels was almost equal.

2. Days to maturity

The estimates of genotypic (5.40%) and phenotypic (5.54) coefficient of variability are comparable but lower than those for days to flowering.

3. Plant height

This character, was found to be coefficient of variability at genotypic (25.34) and phenotypic (25.69) levels were almost identical in magnitude.

4. Number of primary branches per plant

The coefficient of variability at genotypic (23.90%) and phenotypic (24.36%) level were quite different.

5. Number of secondary branches per plant

The coefficient of variability for this trait at genotypic level was 24.47%, whereas it was 26.19% at phenotypic level.

6. Number of flowers per peduncle

The coefficient of variability at genotypic (21.89%) and phenotypic (22.35%) levels were nearly identical.

7. Number of pods/plant

The coefficient of variability for number of pods per plant at genotypic was 23.57% and at phenotypic level it was found to be 23.59%. number of seeds per pod. This character showed the coefficient of variability at genotypic as 20.49% and phenotypic level was 20.86%.

8. Number of seeds per pod

This character showed the coefficient of variability at genotypic as 20.49% and phenotypic level was 20.86%.

9. 100-seed weight (g)

This character showed the coefficient of variability as 29.04% and at phenotypic level it was 29.07%.

10. Grain yield (g)

This grain yield exhibited the coefficient of variability at genotypic level as 23.73%. However, it was 23.75% at phenotypic level.

11. Biomass score (g)

The coefficient of variability observed at genotypic level for biomass score was 23.22%, whereas it was 23.24 at phenotypic level.

12. Harvest index (%)

The coefficient of variability for harvest index at genotypic and phenotypic were 22.65% and 22.75% levels, respectively.

4.2.3 Genetic divergence

Various parameters related to genetic divergence like grouping of genotypic into clusters, intra and inter cluster distances and mean of different characters across the clusters have been estimated according to Rao (1952). The parameter-wise results are presented below:

Table 4.8 Distribution of 100 germplasm lines of lentil in different clusters.

Cluster	No. of germplasm lines	Name (symbol) of germplasm lines
I	9	L63, L68, L70, L71, L75, L81, L82, L89, L91.
II	15	L2, L29, L30, L31, L32, L40, L42, L47, L48, L49, L61, L62, L64, L90, L92.
III	5	L55, L79, L80, L85, L86.
IV	14	L6, L12, L15, L24, L26, L34, L35, L36, L38, L45, L46, L50, L53, L59.
V	4	L65, L67, L74, L76.
VI	7	L60, L69, L77, L83, L93, L94, L99.
VII	5	L72, L73, L87, L88, L100.
VIII	21	L1, L3, L4, L5, L8, L9, L10, L11, L13, L14, L16, L17, L18, L19, L22, L28, L41, L56, L57, L58, L97.
IX	13	L7, L20, L21, L27, L37, L39, L43, L44, L51, L54, L66, L78, L84.
X	7	L23, L25, L33, L52, L95, L96, L98.

4.2.3.1 Clustering of genotypes

On the basis of divergence, 100 germplasm and varieties under study have been grouped in 10 clusters (Table 4.8). The cluster VIII has the maximum lines (21), followed by the cluster II (15), cluster IV (14), cluster IX (13), Cluster I (9), Cluster X, VI (7 each), cluster VII, cluster III (5 each), cluster V (4) genotypes.

4.2.3.2 Intra and inter cluster distances

The divergence within the cluster (intra-cluster) indicates the divergences among the genotypes falling in that particular cluster. On the other hand, the inter-cluster divergence suggests the distance (divergence) between the genotypes of different clusters. The intra-and inter-cluster distances are given in (Table 4.9).

Table 4.9 Intra and inter cluster distance among 10 clusters of 100 germplasm lines of lentil.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	2.234	2.801	3.096	3.958	3.431	3.312	4.471	3.333	3.020	3.676
II		2.239	4.483	3.220	4.134	3.045	5.377	2.895	2.501	3.251
III			2.145	4.349	4.019	5.287	3.576	3.999	3.299	4.520
IV				2.650	4.950	4.489	5.871	2.732	3.884	3.170
V					2.598	5.612	3.028	4.310	4.100	4.981
VI						2.392	6.344	3.186	3.872	3.683
VII							2.273	5.125	4.535	5.977
VIII								2.176	3.525	2.594
IX									1.854	2.984
X										2.792

The result given in (Table 4.9) suggest that the distance among the germplasm lines falling in the cluster I is minimum. On the other hand, the divergence among the lines falling in the cluster VII, VIII, X, has been found maximum, but almost of identical magnitude. The lines falling II, III V, IV, V, IX showed moderate divergence. The cluster VII, X exhibited maximum inter cluster divergence. The distance between the cluster III and IV, III and V, III and VI, III and VII, III and VIII, III and IX, III and X, II and VI have been moderate, but almost of identical magnitude, other cluster combinations exhibited low genetic divergence.

4.2.3.3 Cluster-wise mean

The mean values of each character across the clusters have been worked out and the same have been given in (Table 4.10). The character–wise interpretation results are given below:

1. Days to flowering

A wide range in the mean values of this character across the clusters has been observed. The cluster, VI and IV encompassed the germplasm lines having minimum (64.64) and maximum (91.93) mean number of days to flowering, respectively.

2. Days to maturity

For this developmental character moderate range in the mean across the cluster has been noticed. The cluster IX and IV exhibited minimum (17.92) and maximum (131.04) days to maturity, respectively.

3. Plant height

The clusters formed among 100 genotypes were found quite divergent in respect of plant height. The cluster IX (28.27 cm) and VIII (47.79 cm) generally included dwarf and tall genotypes, respectively.

4. Number of primary branches

A narrow range in the mean values of this character across the ten clusters has been observed. The cluster II and I encompassed the germplasm lines having minimum (1.99) and maximum (3.22) number of primary branches, respectively.

Table 4.10 Cluster wise mean values of 12 characters of 100 germplasm lines of lentil.

S.N.	Characters	I	II	III	IV	V	VI	VII	VIII	IX	X
1.	Days to flowering	71.61	72.90	79.80	91.93	70.00	64.64	73.10	84.10	68.54	86.43
2.	Days to maturity	124.61	118.43	125.00	131.04	129.75	120.29	125.80	129.90	117.92	127.36
3.	Plant height (cm)	34.89	36.83	37.40	35.36	42.88	40.43	36.20	47.79	28.27	30.00
4.	No. of pri. branches	3.22	1.99	3021	2.26	2.91	2.44	2.62	2.14	2.26	2.44
5.	No. of sec. branches	9.03	8.72	13.38	10.69	9.70	8.33	10.20	9.86	13.35	11.97
6.	No. of fl./peduncle	1.99	2.32	2.03	3.10	2.00	2.34	2.00	2.20	2.05	1.94
7.	Pods/plant	137.44	117.70	198.64	133.45	131.73	96.63	176.76	124.13	143.13	105.61
8.	No. of seed/pod	1.62	1.35	1.63	1.32	1.34	1.97	1.79	1.65	1.50	1.53
9.	100-seed weight (g)	4.66	3.94	3.49	2.69	5.16	2.87	4.37	2.30	3.26	1.72
10.	Grain yield (g)	6.19	5.35	8.24	5.76	7.88	4.23	10.32	5.79	6.02	4.26
11.	Biomass score (g)	22.17	20.33	29.93	20.51	44.65	13.79	54.10	22.39	25.84	18.76
12.	Harvest index (%)	28.47	27.03	27.74	29.13	17.44	30.48	18.96	26.96	24.26	23.48

5. Number of secondary branches

The cluster VI and III encompassed the germplasm lines having minimum (8.33) and maximum (13.38) mean number of secondary branches per plant.

6. Number of flowers per peduncle

The cluster X and IV encompassed the germplasm lines having minimum (1.94) and maximum (3.10) mean number of flower per peduncle, respectively.

7. Number of pods per plant

For this character, the ten clusters exhibited tremendous divergence, that is, from 96.63 pods per plant in cluster VI to 198.64 pods per plant in cluster III. It is interesting to mention here that the cluster VI having genotypes with minimum number of pods per plant also had minimum days to flowering, minimum number of secondary branches and lowest biomass score. On the other hand the cluster III having genotypes with maximum number of pods per plant also had maximum number of secondary branches and vice-versa.

8. Number of seeds per pod

The cluster IV and VI encompassed the germplasm lines having minimum (1.32) and maximum (1.97) number of seeds per pod, respectively.

9. 100-seed weight (g)

Widest range in the mean values of this character among the clusters was observed. The cluster X and V exhibited few (1.72) and large (5.16) seeded germplasm lines contained in these clusters, respectively.

10. Grain yield (g)

Widest range in the mean values of this character among the clusters was observed. The cluster V and VII exhibited low (4.23) and high (10.32) grain yield per plant by the germplasm lines contained in these clusters, respectively.

11. Biomass score

For this character the ten clusters exhibited tremendous divergence that is from 13.79 biomass score in cluster VI to 54.10 biomass score in cluster VII. It is interesting to mention here that the cluster VI had genotypes with minimum grain yield and biomass score, on the other hand cluster VII had genotypes with maximum grain yield and biomass score.

12. Harvest index

A wide range in the mean values of this character across the ten clusters has been observed. The cluster V and VI encompassed the germplasm lines having minimum (17.44) and maximum (30.48) harvest index, respectively.

4.2.4 Heritability

The heritability in broad sense was worked out with the help of variability estimated through analysis of variance of the experiment. These estimates have been presented in (Table 4.4).

The estimates of heritability given in (Table 4.11) exhibited a wide range in the values of this parameter. The lowest and highest estimates were recorded with the character biomass score and 100-seed wt.(g) respectively. The characters, which exhibited heritability more than 80% days to flowering, days to maturity, plant height number of flower per peduncle, number of seed per pod, and 100 seed weight, other characters gave heritability values between 75% to 40%.

4.2.5 Genetic Advance

The genetic advances in absolute and in percentage of mean have been estimated and the values have been given in (Table 4.12).

For this parameter also a great range has been observed from 10.60% to biomass score to 51.50% for plant height. The characters number

of pods per plant (18.13%), grain yield (25.00%), number of seeds/pod (26.62%), days to flowering (26.00%), number of secondary branches (27.00%) gave medium values, whereas days to maturity (10.87%), biomass score (10.60%), harvest index (12.31%) gave low estimates of the parameter. 100-seed weight (36.76%), number of flower per peduncle (38.30%), number of primary branches (40.16%) and plant height (51.50%) gave higher values, of genetic advance in percentage of mean.

Table 4.11 Estimate of heritability (broad sense) and genetic advance in respect of 12 characters in lentil.

S.no.	Character	Mean	Heritability %	Genetic advance	Genetic advance in % of mean
1.	Days to flowering	77.84	87.0	20.24	26.00
2.	Days to maturity	125.00	83.0	13.59	10.87
3.	Plant height (cm)	37.65	80.0	19.39	51.5
4.	No. of pri. Br.	2.39	77.0	2.96	40.16
5.	No. of sec. br.	10.41	67.0	2.90	27.0
6.	No. of flower/peduncle	2.27	85.8	0.87	38.3
7.	Pods/plant	131.57	69.9	23.86	18.13
8.	No. of seed/pod	1.54	85.6	0.41	26.62
9.	100 seed wt.(g)	3.21	89.9	1.18	36.76
10.	Grain yield (g)	6.00	49.9	1.50	25.00
11.	Biomass score (g)	24.24	39.9	2.57	10.60
12.	Harvest index	26.32	45.2	3.24	12.31

4.2.6 Correlation

Since 100 germplasm lines on which correlation studies are based, were grown in replicated fashion, the correlation coefficient has been worked out at genotypic, phenotypic and environmental level among twelve quantitative characters, including seed yield under the study. The values of the correlation coefficient have been presented in (Table 4.12, 4.13 and 4.14) and the results are described below:

Table 4.12 Genotypic correlation among 12 characters recorded in 100 germplasm lines of lentil.

Characters	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000	0.639**	0.201*	-0.030	0.128	0.256*	0.072	-0.100	-0.402**	-046	-0.124	0.124
2		1.000	0.322*	0.140	0.038	0.136	0.019	-0.012	-0.233*	0.101	0.047	0.059
3			1.000	-0.104	-0.288*	-0.030	-0.063	0.221*	-0.182	-0.024	-0.031	0.033
4				1.000	0.147	-0.098	0.180	0.160	0.326*	0.190	0.202*	-0.103
5					1.000	-0.087	0.313*	0.057	-0.185	0.162	0.109	-0.026
6						1.000	-0.101	-0.252*	-0.065	-0.114	-0.144	0.104
7							1.000	0.083	0.261*	0.655**	0.502**	-0.057
8								1.000	-0.139	0.099	0.086	-0.017
9									1.000	0.429**	0.420**	-0.147
10										1.000	0.777**	-0.030
11											1.000	-0.612**
12												1.000

* Significant at 5% level

** significant at 1% level

Table 4.13 Phenotypic correlation among 12 characters recorded in 100 germplasm lines of *lentil*.

Characters	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000	0.618**	0.205*	-0.045	0.142	0.241*	0.074	-0.090	-0.394**	-0.47	-0.123	0.123
2		1.000	0.325*	0.139	-0.003	0.154	0.019	0.011	-0.226*	0.105	0.045	0.062
3			1.000	-0.107	-0.272*	-0.022	-0.060	0.220*	-0.178	-0.028	-0.032	0.038
4				1.000	0.116	-0.082	0.174	0.153	0.317*	0.197	0.198	-0.100
5					1.000	-0.110	0.296*	0.034	-0.167	0.177	0.100	-0.025
6						1.000	-0.099	-0.233*	-0.063	-0.123	-0.141	0.104
7							1.000	0.082	0.262*	0.668**	0.501**	-0.057
8								1.000	-0.134	0.111	0.083	-0.015
9									1.000	0.428*	0.420*	-0.146
10										1.000	0.781**	-0.017
11											1.000	-0.612**
12												1.000

Table 4.14 Environmental correlation among 12 characters recorded in 100 germplasm lines of *lentil*.

Character	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000	0.020	0.374*	-0.593**	0.462*	-0.225*	0.667**	0.222*	0.584**	-0.101	-0.154	0.059
2		1.000	0.404*	0.109	-0.502**	0.540**	0.125	0.151	0.116	0.273*	-0.088	0.216*
3			1.000	-0.179	-0.108	0.197	0.355*	0.201*	0.261*	0.294*	-0.289*	0.370*
4				1.000	-0.272*	0.310*	-0.496*	-0.014	-0.406*	0.154	0.107	0.009
5					1.000	-0.418*	0.359*	-0.251*	0.427*	-0.204*	-0.101	-0.032
6						1.000	-0.051	0.186	-0.006	0.245*	-0.039	0.169
7							1.000	0.110	0.603**	0.015	0.010	0.006
8								1.000	0.208*	-0.052	-0.188	0.068
9									1.000	-0.028	-0.093	0.051
10										1.000	-0.091	0.684**
11											1.000	-0763**
12												1.000

The high and positive correlation coefficient of days to flowering with days to maturity, plant height and number of flower per peduncle; days to maturity with plant height; plant height with number of seed per pod; number of primary branches with pods per plant; number of flowers per peduncle with harvest index; pods/plant with 100-seed weight, grain yield and biomass score; number of seed per pod with grain yield; and 100-seed weight with grain yield and harvest index were observed, on the other hand, high and negative association was recorded between few character combinations (days to flowering with 100-seed weight; days to maturity with 100 -seed weight; plant height with number of secondary branches number of flower per peduncle with number of seed per pod biomass score with harvest index). It is interesting to mention here that seed yield per plant has high and positive association with number of pods per plant, 100-seed weight and biomass score. Therefore, improvement in yield is expected through selection for the above component characters.

4.2.7 Path analysis

The path coefficient analysis was carried out to partition, the observed total genotypic, phenotypic and environmental correlation between seed yield per plant and its eleven components in to direct and indirect effects. These estimates of direct and indirect effects and correlation of eleven characters with seed yield per plant at genotypic, phenotypic and environmental levels are given in (Table 4.15) and the results are described below character wise.

Table 4.15 Direct (under lines values on the diagonal) and indirect effect of 11 characters on seed yield per plant in 100 germplasm lines of lentil at genotypic level.

Characters	Days to flowering	Days to maturity	Plant height (cm)	No. of pri. Branches	No. of sec. branches	No. of fl./per peduncle	Pods/plant	No. of seed/pod	100 seed weight (g)	Biomass score (g)	Harvest index (%)	Phenotypic correlation with grain yield
Days to Flowering	0.015	0.005	0.002	-0.004	0.005	-0.002	0.008	-0.001	0.022	-0.138	0.085	-0.047
Days to Maturity	0.009	0.007	0.004	-0.002	0.002	-0.001	0.002	0.005	-013	0.052	0.040	0.105
Plant height (cm)	0.003	0.002	0.011	0.001	-0.012	-0.006	-0.007	0.002	-0.010	-0.035	0.023	0.028
No. of pri. Branch	0.009	0.001	-0.001	-0.013	0.006	0.001	0.020	0.002	0.018	0.224	-0.070	0.197
No. of sec. Branch	0.002	0.008	-0.003	-0.002	0.042	0.001	0.035	0.001	-0.010	0.121	-0.018	0.177
No. of flower/peduncle	0.004	0.001	-0.009	0.001	-0.004	-0.009	-0.011	-0.003	-0.004	-0.160	0.071	-0.123
Pods/plant	0.001	0.010	-0.001	-0.002	0.013	0.001	0.112	0.001	0.014	0.558	-0.039	0.668
No. of seed/pod	-0.001	0.002	0.002	-0.002	0.002	0.002	0.009	0.010	-0.008	0.096	-0.011	0.111
100 seed wt. (g)	-0.006	-0.002	-0.002	-0.004	-0.008	0.001	0.029	-0.001	0.055	0.468	-0.101	0.429
Biomass score (g)	-0.002	0.004	0.003	-0.003	0.005	0.001	0.056	0.001	0.023	1.112	-0.419	0.777
Harvest index (%)	0.002	-0.006	-0.007	0.001	-0.001	-0.001	-0.006	-0.008	-0.008	-0.681	0.685	-0.030

Residual effect = 0.0407

Table 4.16 Direct (under line values on the diagonal) and indirect effects of eleven characters on seed yield per plant in 100 germplasm lines of lentil at phenotypic level.

Character	Days to flowering	Days to maturity	Plant height (cm)	No. of pri. branches	No. of sec. branches	No. of flower/peduncle	Pods/plant	No. of seed/pod	100- seed weight	Biomass score (g)	Harvest index (%)	Phenotypic correlation with grain yield
Days to flowering	0.010	0.006	0.001	-0.002	0.005	-0.002	0.009	-0.001	-0.019	-0.137	0.084	-0.046
Days to Maturity	0.006	0.010	0.002	-0.001	0.001	-0.001	0.002	0.001	-0.001	0.050	0.042	0.101
Plant height (cm)	0.002	0.003	0.007	0.001	-0.010	-0.003	-0.007	0.002	-0.009	-0.036	0.026	-0.024
No. of pri. branches	0.006	0.001	-0.001	-0.009	0.004	0.001	0.020	0.001	0.015	0.221	-0.069	0.190
No. of sec. branches	0.001	0.006	-0.002	-0.001	0.035	0.001	0.034	0.001	-0.008	0.112	-0.017	0.160
No. of flower/peduncle	0.002	0.002	-0.005	0.001	-0.004	-0.008	-0.011	-0.002	-0.003	-0.157	0.071	-0.114
No. of pods/plant	0.001	0.006	0.001	-0.002	0.010	0.001	0.115	0.001	0.013	0.558	-0.039	0.665
No. of seed/pod	-0.001	0.001	0.002	-0.001	0.001	0.002	0.009	0.010	-0.007	0.093	-0.010	0.099
100 seed wt. (g)	-0.004	-0.002	-0.001	-0.003	-0.006	0.001	0.030	-0.001	0.048	0.467	-0.100	0.429
biomass score (g)	-0.001	0.002	0.001	-0.002	0.004	0.001	0.058	0.001	0.020	1.112	-0.419	0.781
Harvest index (%)	0.001	0.001	-0.003	0.001	-0.001	-0.001	-0.007	-0.005	-0.007	-0.681	0.685	0.017

Residual effect = 0.0414

1. Days to flowering and seed yield

Days to flowering did not show appreciable association in either direction. However, its direct effect (0.010) has been found positive. Also, it showed considerable positive effect on seed yield via harvest index (0.084). However, via days to maturity (0.006), plant height (0.001), number of secondary branches (0.005), pods per plant (0.009) it showed positive indirect effect, but of low magnitude. Similarly, via number of primary branches (-0.002), number of flower per peduncle (-0.002), number of seed per pod (-0.001), 100 seed weight (-0.019) and biomass score (-0.137), it showed negative but marginal indirect effect.

2. Days to maturity and seed yield

This character showed positive correlation with seed yield, but of quite low magnitude (0.101). However, it has negative and marginal effect on yield via number of primary branches (-0.001), number of flower per peduncle (-0.001), 100 seed weight (-0.001). On the other hand, its positive and marginal indirect effect on seed yield via plant height (0.002), number of secondary branches (0.001), pods per plant (0.002), number of seed per pod (.001), biomass score (.050) and harvest index (0.042) has been noted.

3. Plant height and seed yield

It has negative and marginal phenotypic correlation with seed yield (-0.024). However, it has positive direct effect (0.007). It also has very low and positive indirect effect via only five characters (days to flowering, days to maturity, number of primary branches, number of seed per pod and harvest index) and negative effect via other characters (number of secondary branches, number of flower per peduncle, pods per plant, 100 seed weight, biomass score). Number of primary branches and

seed yield: It has positive but low phenotypic correlation with seed yield (0.190). Also, it has very marginal negative direct effect on seed yield (-0.009). However, it has indirect and high positive effect on yield via pods per plant (0.020), 100 seed weight (0.015) and biomass score (0.221) and indirect and high negative effect via harvest index (-0.069).

4. Number of secondary branches and seed yield

It has high (0.160) and positive phenotypic association with seed yield. However, it has direct effect of positive magnitude (0.035). Indirectly it contributed positively to seed yield via pods per plant (0.034) and biomass score (0.112), but via other 4 characters it had negative and low magnitude effect on seed yield.

5. Number of flower per peduncle and seed yield

It has negative but (-0.114) phenotypic association with seed yield. However, it had direct effect of negative and low magnitude (-0.008). Indirectly it contributed positively to seed yield via harvest index (0.071), but via other five characters it had negative effects on seed yield and biomass score shown highly negative magnitude (-0.157).

6. Pods per plant and seed yield

It has very high (0.665) and positive phenotypic association with seed yield. However, it had direct effect of also high magnitude (0.115). Indirectly it contributed very high positively to seed yield via biomass score (0.558) on the other hand only one character harvest index shown highly negative indirect effect on seed yield (-0.039).

7. Number of seed per pod and seed yield

It has positive and phenotypic association with seed yield (0.010). Also it has low (0.099) direct effect on seed yield. Indirectly, it has effect on yield via days to maturity and 100 seed weight. Indirect negative effect on yield has been noted via days to flowering (0.001), number of primary branches (-0.001), 100-seed weight (-0.007) and harvest index (-0.010), on the other hand, via biomass score it has highly positive (0.093) effect.

8. 100-seed weight and seed yield

It has also high positive correlation with seed yield (0.429). It has also high (0.048) direct effect on seed yield. But via pods per plant (0.030) and biomass score (0.467) it has highly positive indirect effect. However via as many as seven characters (days to flowering, days to maturity, plant height, number of primary branches, number of secondary branches, number of seed per pods and harvest index) it has indirect negative effect of low magnitude on seed yield.

9. Biomass score and seed yield

Biomass score shown positive phenotypic association of very high magnitude with seed yield (0.781). It has also high (1.112) direct effect on seed yield. Pod per plant (0.058) and 100 seed weight (0.020) shown high positively indirect effect. On the other hand harvest index shown very high negative magnitude (-0.419) has been noted.

10. Harvest index and seed yield

This character showed positive correlation with seed yield but of quite low magnitude (0.017). However, it has very high (0.685) direct effect on seed yield. Indirectly it contributed positively to days to flowering, days to maturity, and number of primary branches. But via

other five characters it had negative low magnitude and harvest index shown very high negative magnitude (-0.681) effect on seed yield.

4.3 Molecular Markers

For this study a set of 60 germplasm lines as listed in (Table 3.3) have been used for RAPD analysis and similarity index, as proposed by Jaccard's (1908), has been worked out. The result on different aspects of molecular analysis has been presented below:

4.3.1 Rapid amplification of polymorphic DNA

The total genomic DNA was extracted from 5 gm fresh leaves of each of the 60 genotypes of lentil. The quantification of the DNA was done after subjecting the DNA to RNase treatment.

The DNA content ranged from 1.0-4.0 µg/µl. The purified and quantified DNA was stored at -20^0C in small aliquots in TE buffer (10 mM Tris Hydrochloride and 1 mM sodium EDTA, pH 8.0). The DNA of each of the genotypes was subjected to polymerase chain reaction using decamer random primers obtained from Operon Technologies In, USA. The PCR protocol was first optimized in terms of concentration of template DNA, Taq DNA polymerase, and $MgCl_2$ concentration. Varying the concentrations of template DNA from 25 ng to 100 ng in a reaction volume of 25 µl revealed that 25 ng of DNA gave maximum number of reproducible bands and thus was considered ideal and used subsequently in all analysis. A titration of different concentrations of taq DNA polymerase and $MgCl_2$ showed that 0.5 unit of taq DNA polymerase and 1.5 mM $MgCl_2$ gave optimum results for PCR amplification. A higher or lower concentration resulted in either sub-optimal or complete lack of amplification. The final amplification assay contained 25 ng genomic

DNA, - 0.5 units Taq DNA polymerase, 0.5ml dNTPs, and 2.5mM 10x PCR assay buffer, 1.0ml primer 18ml sterile double distilled water in a reaction volume of 25 µl.

The amplification reaction was carried out using thermocycler machine following temperature profile: 94^{0}C for 5 minutes, 37^{0}C for 1 minute and 72^{0}C for 2 minutes, 37^{0}C for 1 minute and 72^{0}C for 2 minutes with a final extension step of 72^{0}C for 7 minutes.

4.3.2 Primer selection and survey

Primer survey was carried out using 200 primers from OPA, OPB, OPC, OPM, OPM, OPP, OPU, OPS, OPV, OPW, series of Operon Technologies Inc., USA and the data are given in (Table 4.17, 4.18, 4.19):

Out of 200 primers screened for amplification of DNA of 60 lentil genotypes based on the reproducibility of the bands, consistency in result and polymorphic behavior, about 80 resulted in either sub-optimal or non-distinct amplification products. Therefore, these were discarded and remaining 120 used for twice PCR amplification. However, among these 120 were primers, the amplification obtained using 100 primers was not polymorphic in behavior. Therefore only 20 primers were used for PCR amplification. However, among these 20 primers, the amplification obtained using 10 primers (OPA1, OPB7, OPC4, OPV6, OPV14, OPW3, OPW8, OPW9, OPW15) was not consistent in all the 60 samples. Therefore, the results of amplification patterns from these 10 primers were not used in the statistical analysis. The final numerical taxonomic analysis included the results from only rest of 10 primer amplifications.

Table 4.17 DNA amplification pattern of RAPD primers in 60 lentil genotypes

Primers		OPA19			OPB 8					OPB 18					OPN 20					OPU 2		
	Genotype / Band	1	2	3	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3
1.	Sehore 74-3	-	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-
2.	LH90-57	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-
3.	L830	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-
4.	JLS-1	-	+	+	+	+	-	+	+	+	-	-	+	+	-	+	+	+	+	+	+	-
5.	LC 93-5-3-3-1	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-
6.	L 4149	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-	-
7.	E-153	-	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	-	-	+
8.	L 1304	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	-	-	-
9.	L 4147	-	-	-	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	-	-	-
10.	L 4378	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-
11.	L 4384	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	-	-	-
12.	L 435	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-
13.	SKL 259	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-
14.	10-2-B-2	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-
15.	L 3685	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
16.	L4387	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
17.	LC 74-1-5-1	-	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	-
18.	Pusa4	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
19.	PL 639	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	-

Contd.

20.	L 4603	-	-	-	-	+	-	+	+	-	-	+	+	+	+	+	-	+	+	+	-	-
21.	L 4602	-	+	+	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	-	-
22.	Fasciated Mutant	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-
23.	Dwarf Mutant	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-
24.	8-1	-	+	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-
25.	Globe mutant	-	+	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-	-
26.	10-3Y-26 N	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-
27.	19-B-5G	-	+	+	+	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-
28.	19-B-5 N	-	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+
29.	L4605	+	+	-	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	-
30.	L 6183	-	+	+	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	+	-	-
31.	PL 406	-	+	-	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-
32.	LC 68-17-4-1	-	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-
33.	EC 383090	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-
34.	EC 383087	-	+	+	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	-
35.	P 2221	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	-	-
36.	P 33159	-	+	+	-	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	-	-
37.	14-1-Y-50	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	-	-
38.	L 4076	-	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-
39.	Precoz	-	+	+	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	+	-	-

Contd.

40.	MC6	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	+	+	+	-	-
41.	MC 1	-	+	-	+	+	-	-	+	+	-	-	-	+	+	+	+	-	+	-	-	-
42.	25-26	-	-	+	+	+	-	-	+	-	-	-	-	-	+	-	+	-	+	+	-	-
43.	LC68-17-3-5	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-
44.	8-3	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-
45.	ICARDA PROMISING	-	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	-
46.	EC 383084	-	+	+	+	+	-	-	+	+	-	+	-	-	+	+	+	+	+	+	+	-
47.	P 22127	+	+	+	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-
48.	RL-1	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+
49.	P 22107	-	+	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-
50.	L 5256	-	+	-	-	+	-	-	+	+	-	-	+	+	+	+	-	+	+	+	-	-
51.	PKVL1	-	+	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	-	-
52.	P22115	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-
53.	10-3Y-26g	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-
54.	FLIP96-51	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
55.	PL5	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	-	-
56.	DPL21	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	-	-
57.	DPL15	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	-	-
58.	DPL62	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+	+	-	+
59.	LPL81	+	+	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	+	-	-
60.	L5120	-	-	-	-	+	-	-	+	+	-	+	-	-	+	+	-	+	+	+	-	-

Contd.

S.No.	Primers	OPU 6			OPU 14					OPV 12										OPV 20		OPW 13		
	Genotype / Band	1	2	3	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	1	2	1	2	3
1.	Sehore 74-3	-	-	+	-	+	+	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+
2.	LH90-57	-	-	-	-	+	+	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-	+	+
3.	L830	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
4.	JLS-1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+
5.	LC 93-5-3-3-1	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+
6.	L 4149	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
7.	E-153	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
8.	L 1304	+	+	+	+	-	+	-	-	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+
9.	L 4147	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
10.	L 4378	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.	L 4384	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
12.	L 435	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
13.	SKL 259	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
14.	10-2-B-2	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
15.	L 3685	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+
16.	L4387	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+
17.	LC 74-1-5-1	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
18.	Pusa4	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+

Contd.

19.	PL 639	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+
20.	L 4603	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21.	L 4602	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+
22.	Fasciated Mutant	+	+	+	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
23.	Dwarf Mutant	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+
24.	8-1	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25.	Globe mutant	-	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
26.	10-3Y-26 N	+	+	+	+	-	+	-	-	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+
27.	19-B-5G	-	-	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+
28.	19-B-5 N	+	+	+	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+
29.	L4605	-	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	+	+	+
30.	L 6183	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
31.	PL 406	+	+	+	-	-	+	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	+	+
32.	LC 68-17-4-1	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	+	-	+	-	+	+	+	+	+
33.	EC 383090	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
34.	EC 383087	+	+	+	-	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	+	+	+	+	+
35.	P 2221	+	+	+	-	-	+	-	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+
36.	P 33159	-	-	-	-	-	+	-	-	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+
37.	14-1-Y-50	-	-	-	-	+	+	+	-	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+
38.	L 4076	-	-	-	-	+	+	-	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+
39.	Precoz	-	-	-	+	+	+	-	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+

Contd.

40.	MC6	-	-	-	-	-	+	-	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+
41.	MC 1	-	-	-	-	-	+	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	+	+
42.	25-26	-	-	-	-	-	+	-	-	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+
43.	LC68-17-3-5	-	-	-	-	-	+	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	+	+
44.	8-3	-	-	-	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+
45.	ICARDA PROMISING	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+
46.	EC 383084	-	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+
47.	P 22127	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
48.	RL-1	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
49.	P 22107	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
50.	L 5256	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+
51.	PKVL1	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
52.	P22115	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	-
53.	10-3Y-26g	-	-	-	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+
54.	FLIP96-51	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+
55.	PL5	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
56.	DPL21	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57.	DPL15	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
58.	DPL62	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+
59.	LPL81	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+
60.	L5120	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+

Table 4.18 Details of presence of specific bands in diverse germplasm of lentil.

Primer	Band	Number of genotypes with this band	Informative Band
OPA19	1	9	0.300
	2	38	1.600
	3	34	1.32
OPB8	1	45	1.500
	2	32	1.066
	3	23	0.766
	4	26	0.866
	5	59	1.966
OPB18	1	39	1.300
	2	18	0.600
	3	17	0.566
	4	44	1.433
	5	46	1.532
OPN20	1	41	1.366
	2	42	1.400
	3	34	1.132
	4	50	1.666
	5	57	1.900
OPU2	1	60	2.000
	2	9	0.300
	3	5	0.166
OPU6	1	32	1.066
	2	47	1.566
	3	40	1.132
OPU14	1	15	0.500
	2	14	0.466
	3	60	2.00
	4	16	0.532
	5	10	0.332
OPV12	1	46	1.532
	2	55	1.832
	3	38	1.266
	4	45	1.050
	5	51	1.700
	6	39	1.300
	7	55	1.832
	8	52	1.732
	9	27	0.900
	10	53	1.766
OPV20	1	30	1.000
	2	46	1.532
OPW13	1	46	1.532
	2	50	1.666
	3	58	1.932

Table 4.19 Random primers used and the polymorphism obtained in 60 germplasm lines of lentil.

Sl.No.	Primers	Base sequence (5' to 3')	Polymorphism obtained		Resolving Power	Molecular weight range (kb)
			Total no. of loci amplified	No. of Polymorphic bands		
1.	OPA19	CAAACGTCGG	3	3	3.032	0.5-1.0
2.	OPB8	GTCCACACGG	5	5	6.164	0.2-1.2
3.	OPB18	CCACAGCAGT	5	5	5.431	0.1-1.1
4.	OPN20	CGTGCTCCGT	5	5	7.652	0.4-1.3
5.	OPU2	CTGAGGTCTC	3	2	2.466	0.4-1.2
6.	OPU6	ACCTTTGCGG	3	3	3.769	0.5-1.0
7.	OPU14	TGGGTCCCTC	5	4	3.830	0.2-1.4
8.	OPV12	ACCCCCCACT	10	10	14.910	0.2-1.6
9.	OPV20	CAGCATGGTC	2	2	2.532	0.7-1.0
10.	OPW13	CACAGCGACA	3	3	5.130	0.5-1.2

PCR amplifications with each of the primer were done twice before scoring for presence and absence of brands. The number of amplification products obtained were specific to each primer and ranged from 2 to 10 with fragment size varying from 100 kb to 1.60 kb. On the other hand observed high resolving power (14.91) with primer OPV 12. The selected primers were polymorphic in nature. This indicated that only 1 % of bands were non-polymorphic, although 20 primers product ed polymorphic bands, there was no single primer that could clearly distinguish all the 60 accessions. Hen the unique bands for each accession were considered it was observed that there were as much as 59 accessions could be kinked to these unique polymorphic bands.

A high degree of polymorphism, in general, was obtained with most of the primers (fig. 7 to 12), specially OPV12(10), OPB8(5), OPB18(5), OPN20(5) indicating a wide range of diversity DNA level also among the material studied. These primers can also be used for cultivars identification and molecular mapping of lentil genome. This can also serve as a reference for integral comparison in future studies. It was interesting to observe that there was no instance of any two accessions sharing all the bands with any primer, which eliminated the possibility of presence of duplicates in the 60 germplasm accessions analysed.

Fig. 7. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPB8. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*

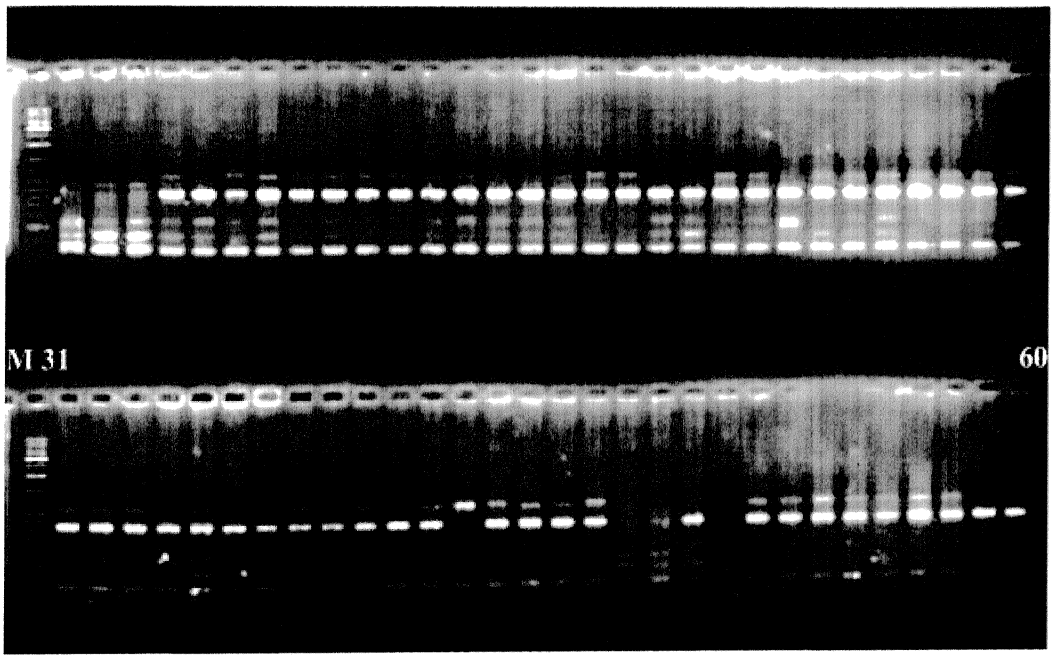


Fig. 8. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPB18. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*

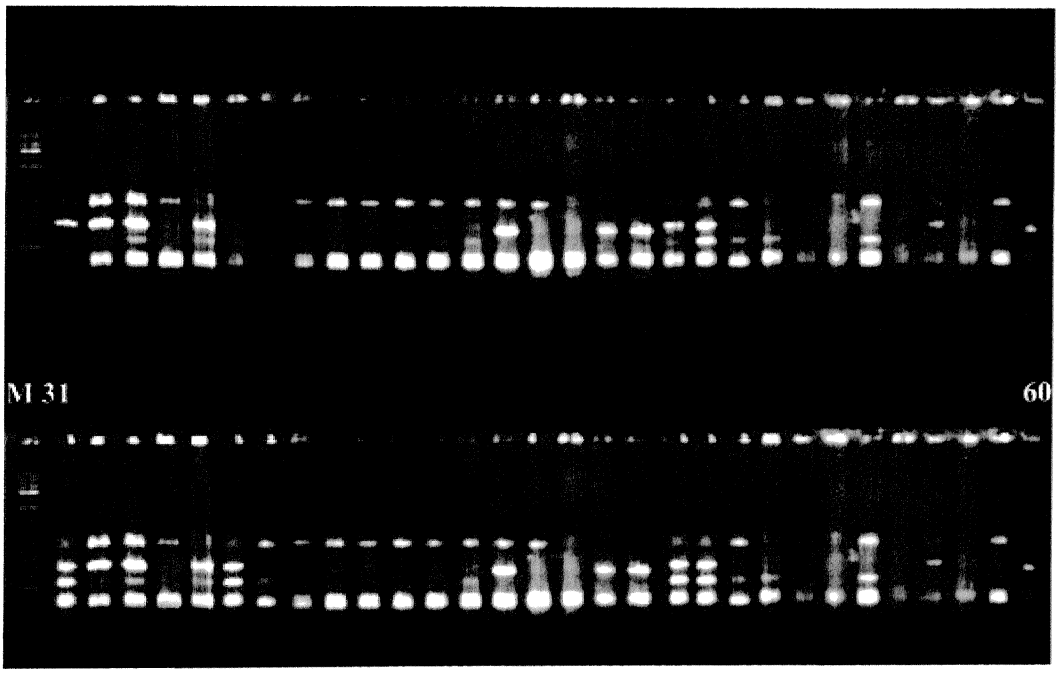


Fig. 9. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPU2. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*

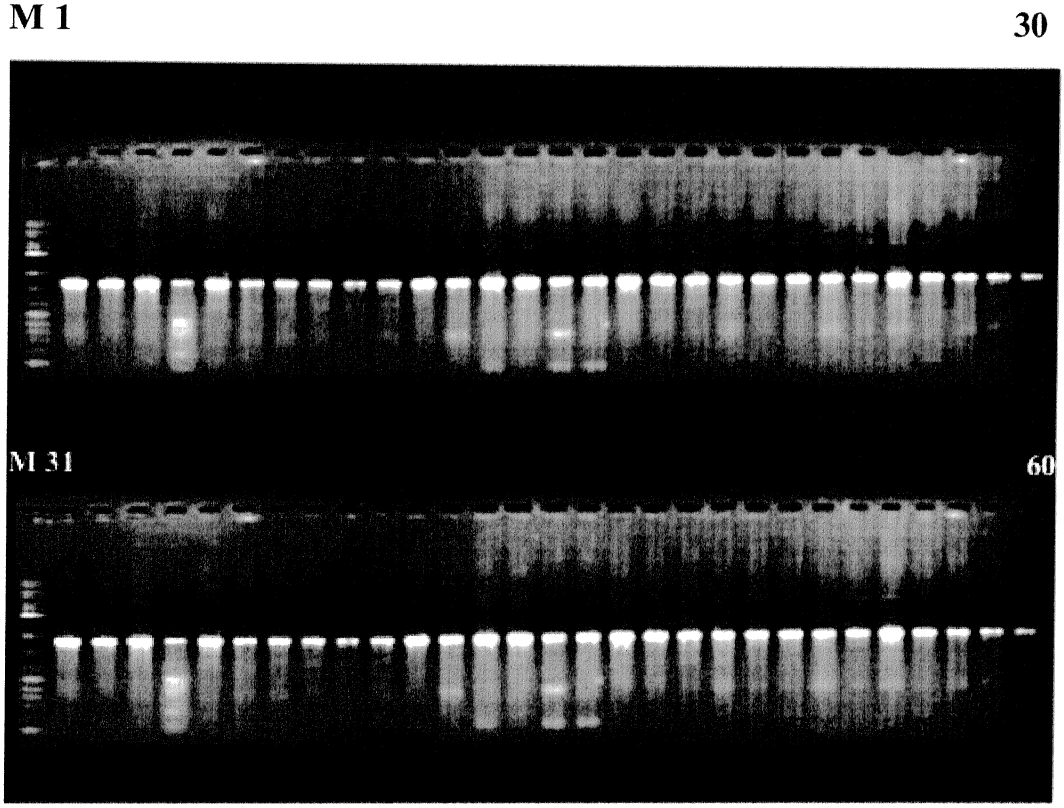


Fig. 10. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPU14. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*

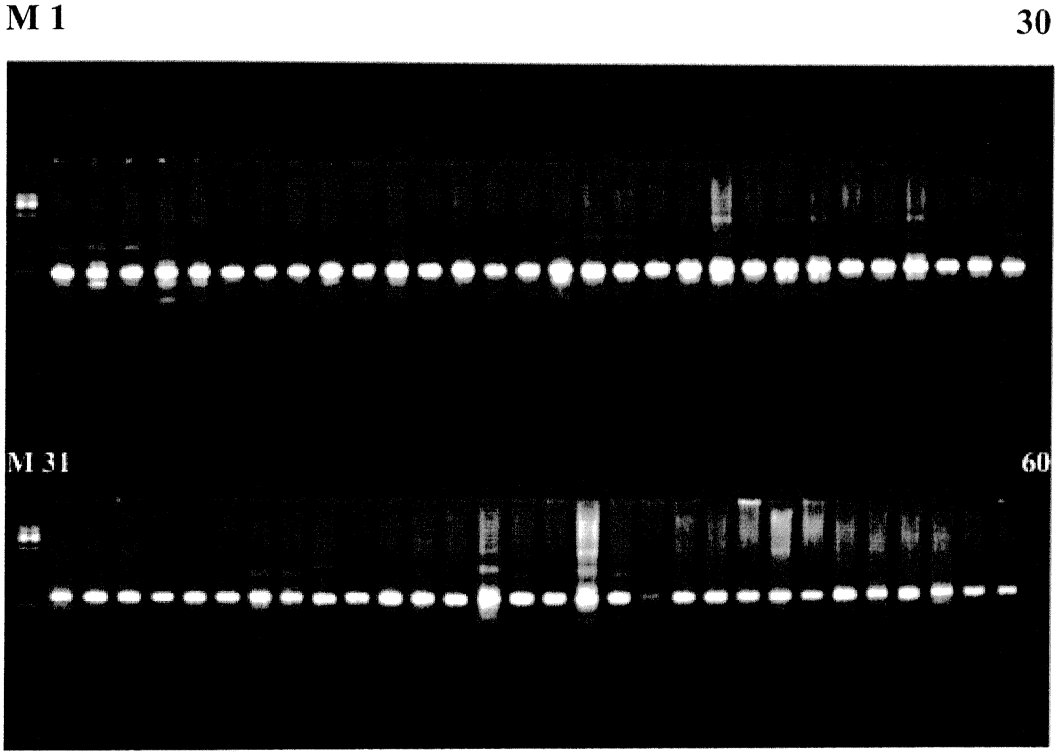


Fig. 11. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPV12. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*

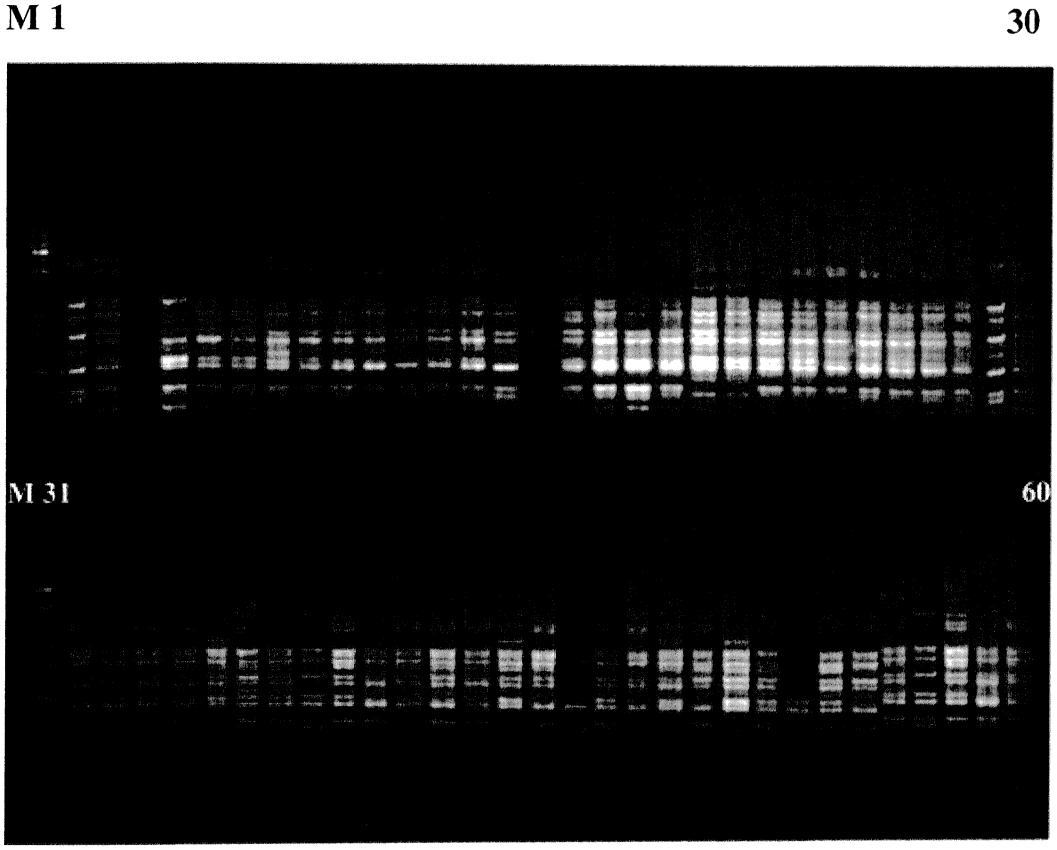
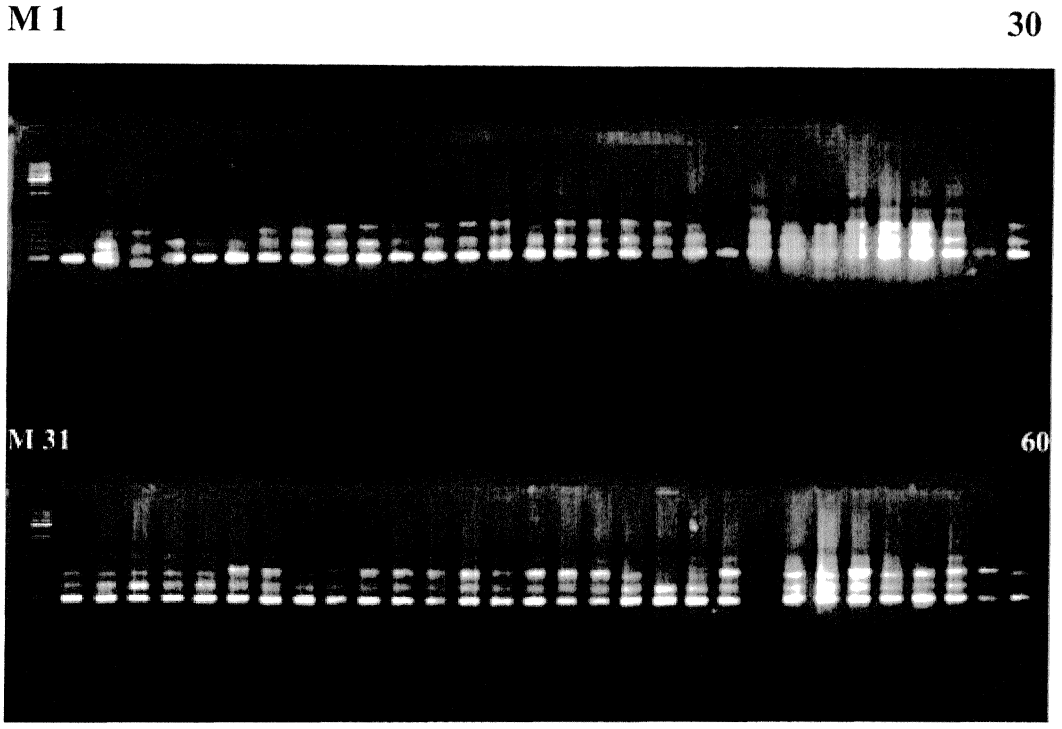


Fig. 12. RAPD amplification of 60 germplasm lines of lentil genotypes with random Operon primer OPW13. Lanes 1 - 60 are 60 germplasm lines as listed serially in (Table 3.3). Lane M is marker A λ DNA digested with *Hind III* and *EcoRI*



4.3.3 *Similarity index*

Banding profiles obtained with 10 primers for 60 genotypes of lentil were analyzed on the basis of presence or absence of the band. Jaccard's similarity coefficient between the 60 lentil genotypes were calculated to analyze the relatedness and the same have been given in Table 4.20. The similarity index values ranged from 0.111-0.975, again indicating the presence of wide range of genetic diversity at molecular level among 60 germplasm lines used in the present study. The most diverse pair showing (0.111) similarity coefficient comprised of accession L15 and L33, whereas maximum closeness (0.906) was observed between L10 and L12. The rest of the accessions showed the similarity coefficient ranging from 0.149 to 0.973. These similarity coefficients were subjected to unweighted pair group method on arithmetic average (UPGMA) using paired matrix values. The total number of bands obtained were 44 out of which 42 bands were polymorphic the dendrogram obtained after cluster analysis is shown in (fig 13). This dendrogram also exhibits variables degree of relationships among the germplasm studied. The dendrogram consisted of six close V knit cluster. However the number of accessions varied from 2(cluster V) to 12 (cluster VI). The cluster VIII having an accession while cluster IV had 8 accessions, cluster IX had 7 accessions cluster III and VII had 5 accession each. The cluster I, II and X comprised of 4 genotypes each.

Fig. 13. Dendrogram of 60 lines of lentil based on DNA polymorphism obtained with 10 random primers

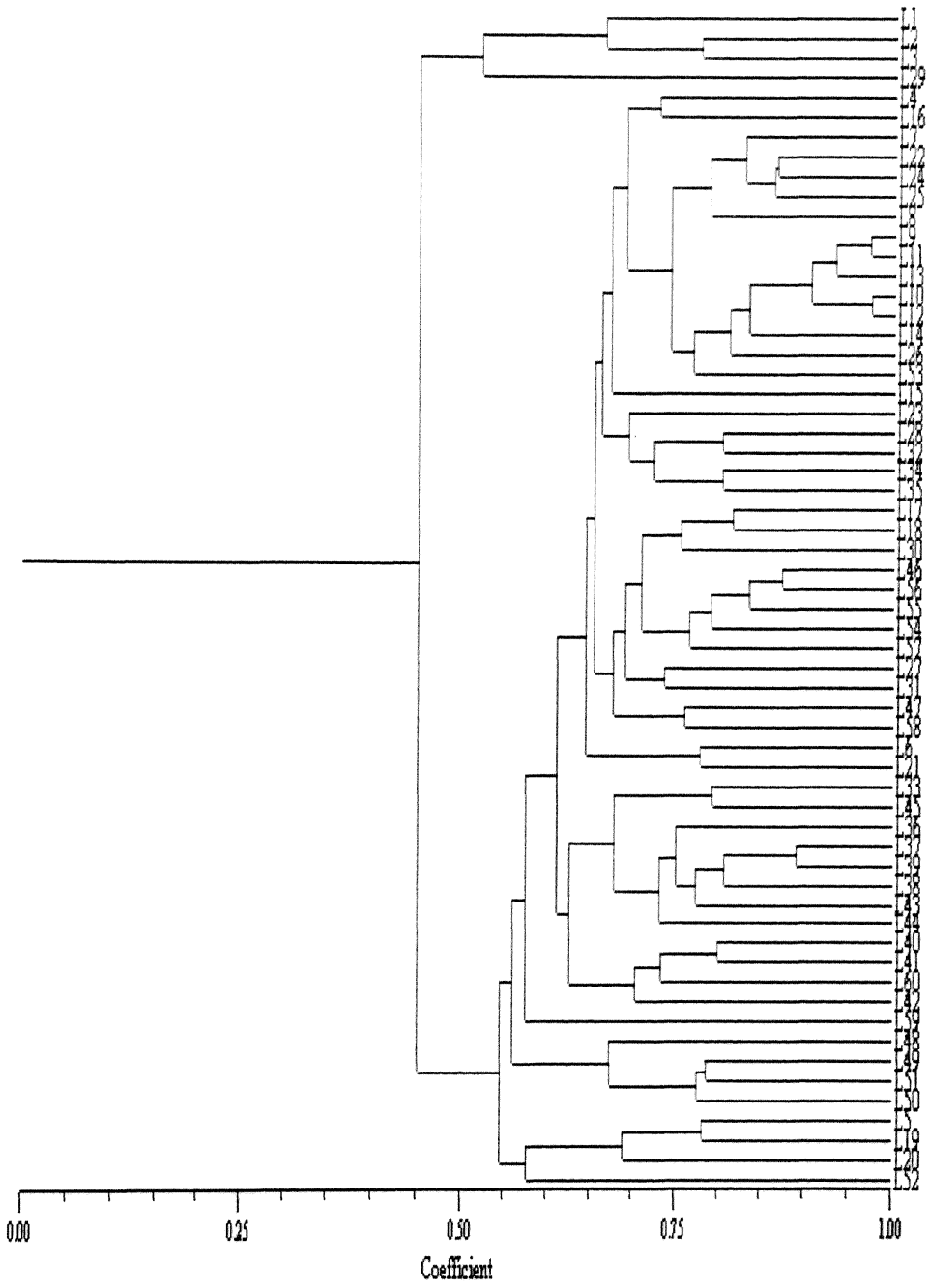


Table 4.20 Jaccard's similarity coefficient of 60 germplasm lines of *lentil* based on polymorphism obtained with 10 random primers.

1.000

0.696 1.000

0.636 0.777 1.000

0.400 0.520 0.448 1.000

0.487 0.545 0.571 0.562 1.000

0.372 0.437 0.456 0.652 0.577 1.000

0.422 0.510 0.440 0.687 0.551 0.750 1.000

0.304 0.428 0.360 0.673 0.469 0.738 0.772 1.000

0.418 0.543 0.468 0.625 0.697 0.608 0.795 0.704 1.000

0.454 0.574 0.500 0.687 0.652 0.673 0.863 0.772 0.926 1.000

0.428 0.555 0.478 0.638 0.674 0.622 0.813 0.681 0.973 0.902 1.000

0.465 0.586 0.510 0.666 0.667 0.652 0.840 0.750 0.950 0.975 0.925 1.000

0.428 0.521 0.478 0.604 0.674 0.622 0.772 0.644 0.923 0.857 0.947 0.877
1.000

0.422 0.574 0.500 0.620 0.617 0.571 0.744 0.625 0.837 0.822 0.857 0.800
0.857 1.000

0.363 0.555 0.446 0.638 0.565 0.553 0.659 0.574 0.744 0.733 0.761 0.711
0.720 0.772 1.000

0.434 0.583 0.480 0.729 0.560 0.549 0.714 0.702 0.723 0.750 0.702 0.765
0.666 0.647 0.568 1.000

0.391 0.510 0.440 0.653 0.490 0.711 0.708 0.772 0.645 0.673 0.625 0.687
0.591 0.607 0.500 0.750 1.000

0.347 0.469 0.428 0.680 0.510 0.785 0.739 0.767 0.604 0.666 0.617 0.645
0.617 0.600 0.520 0.607 0.818 1.000

0.542 0.600 0.631 0.510 0.783 0.634 0.600 0.446 0.682 0.636 0.700 0.651
0.700 0.636 0.581 0.510 0.531 0.555 1.000

0.355 0.510 0.468 0.695 0.697 0.608 0.645 0.595 0.727 0.717 0.704 0.695
0.704 0.645 0.595 0.588 0.580 0.638 0.682 1.000

0.395 0.489 0.478 0.750 0.636 0.780 0.733 0.681 0.666 0.695 0.644 0.711
0.608 0.560 0.541 0.666 0.695 0.652 0.619 0.666 1.000

0.400 0.520 0.420 0.739 0.562 0.727 0.840 0.803 0.813 0.837 0.790 0.866
0.750 0.723 0.638 0.765 0.723 0.717 0.543 0.695 0.750 1.000

0.312 0.460 0.420 0.666 0.562 0.617 0.687 0.673 0.625 0.687 0.638 0.666
0.638 0.588 0.604 0.693 0.588 0.645 0.510 0.659 0.571 0.666 1.000

0.340 0.480 0.415 0.787 0.580 0.739 0.847 0.800 0.744 0.808 0.723 0.787

0.687 0.666 0.620 0.775 0.770 0.765 0.530 0.744 0.840 0.866 0.714 1.000

0.369 0.460 0.392 0.739 0.500 0.688 0.800 0.750 0.733 0.800 0.711 0.777

0.673 0.653 0.638 0.693 0.723 0.717 0.510 0.695 0.711 0.860 0.666 0.866

1.000

0.386 0.479 0.380 0.591 0.586 0.574 0.755 0.666 0.853 0.837 0.829 0.813
0.785 0.755 0.704 0.687 0.612 0.604 0.568 0.652 0.595 0.772 0.591 0.744

0.733 1.000

0.333 0.400 0.333 0.571 0.469 0.659 0.659 0.720 0.562 0.625 0.541 0.604
0.541 0.560 0.510 0.538 0.695 0.727 0.416 0.595 0.608 0.673 0.571 0.723

0.711 0.562 1.000

0.377 0.469 0.372 0.645 0.480 0.630 0.702 0.727 0.673 0.739 0.652 0.717

0.617 0.600 0.652 0.640 0.666 0.695 0.489 0.604 0.583 0.717 0.680 0.693

0.755 0.711 0.727 1.000

0.461 0.558 0.547 0.510 0.500 0.627 0.530 0.510 0.531 0.562 0.543 0.574

0.543 0.530 0.613 0.540 0.562 0.553 0.547 0.469 0.510 0.510 0.510 0.500

0.574 0.531 0.479 0.586 1.000

0.347 0.440 0.400 0.612 0.510 0.704 0.666 0.688 0.638 0.666 0.617 0.168

0.652 0.568 0.149 0.673 0.777 0.733 0.489 0.638 0.652 0.717 0.645 0.765
0.755 0.641 0.727 0.695 0.586 1.000

0.372 0.500 0.425 0.583 0.543 0.600 0.604 0.697 0.681 0.711 0.659 0.727

0.622 0.604 0.586 0.612 0.711 0.666 0.488 0.608 0.586 0.688 0.617 0.702

0.652 0.608 0.738 0.704 0.521 0.704 1.000

0.414 0.545 0.404 0.595 0.458 0.613 0.688 0.674 0.659 0.688 0.674 0.704
0.636 0.583 0.600 0.659 0.652 0.681 0.500 0.520 0.565 0.704 0.666 0.645

0.666 0.622 0.600 0.848 0.646 0.644 0.734 1.000

0.285 0.422 0.291 0.543 0.404 0.581 0.565 0.658 0.333 0.565 0.554 0.577

0.511 0.500 0.111 0.574 0.636 0.707 0.409 0.468 0.511 0.651 0.613 0.595

0.577 0.533 0.619 0.707 0.444 0.590 0.717 0.783 1.000

0.428 0.521 0.446 0.604 0.600 0.659 0.695 0.604 0.666 0.695 0.601 0.711

0.681 0.501 0.541 0.666 0.655 0.688 0.581 0.597 0.541 0.711 0.796 0.653

0.638 0.343 0.541 0.688 0.577 0.688 0.659 0.800 0.658 1.000

0.463 0.521 0.446 0.604 0.531 0.622 0.733 0.574 0.666 0.695 0.681 0.711

0.681 0.591 0.574 0.702 0.659 0.652 0.581 0.562 0.574 0.711 0.638 0.687

0.673 0.630 0.574 0.652 0.543 0.652 0.659 0.756 0.619 0.804 1.000

0.375 0.477 0.369 0.531 0.425 0.619 0.622 0.642 0.521 0.586 0.533 0.565

0.500 0.520 0.468 0.597 0.697 0.690 0.500 0.555 0.500 0.674 0.674 0.652
0.636 0.521 0.642 0.613 0.432 0.613 0.700 0.675 0.702 0.725 0.725 1.000

0.372 0.500 0.367 0.617 0.420 0.636 0.673 0.697 0.574 0.638 0.586 0.617

0.553 0.540 0.520 0.717 0.711 0.666 0.425 0.541 0.553 0.727 0.688 0.702

0.652 0.574 0.622 0.666 0.489 0.704 0.714 0.731 0.717 0.697 0.697 0.789
1.000
0.459 0.560 0.409 0.543 0.466 0.595 0.565 0.581 0.568 0.600 0.581 0.613
0.545 0.531 0.545 0.608 0.674 0.590 0.512 0.468 0.511 0.613 0.543 0.562
0.543 0.533 0.511 0.555 0.547 0.590 0.717 0.660 0.675 0.619 0.619 0.702
0.810 1.000
0.357 0.456 0.326 0.541 0.380 0.666 0.704 0.690 0.565 0.595 0.577 0.608
0.543 0.500 0.479 0.673 0.785 0.697 0.444 0.500 0.577 0.681 0.608 0.695
0.644 0.565 0.613 0.622 0.511 0.697 0.666 0.725 0.710 0.690 0.731 0.783
0.891 0.805 1.000
0.324 0.372 0.266 0.500 0.452 0.512 0.595 0.615 0.684 0.634 0.702 0.650
0.657 0.595 0.536 0.533 0.558 0.511 0.461 0.523 0.465 0.609 0.534 0.555
0.571 0.600 0.500 0.547 0.395 0.549 0.631 0.564 0.540 0.575 0.575 0.567
0.675 0.676 0.666 1.000
0.378 0.386 0.311 0.511 0.500 0.523 0.568 0.585 0.571 0.604 0.585 0.619
0.547 0.500 0.477 0.543 0.604 0.558 0.404 0.466 0.444 0.581 0.545 0.565
0.581 0.534 0.547 0.595 0.441 0.634 0.729 0.615 0.594 0.625 0.666 0.666
0.729 0.735 0.722 0.800 1.000
0.333 0.347 0.276 0.500 0.391 0.585 0.627 0.609 0.595 0.555 0.609 0.568
0.609 0.555 0.500 0.565 0.666 0.619 0.463 0.522 0.500 0.604 0.533 0.622
0.642 0.558 0.650 0.619 0.431 0.700 0.625 0.600 0.578 0.571 0.650 0.694
0.710 0.621 0.750 0.718 0.727 1.000
0.410 0.477 0.369 0.531 0.395 0.581 0.622 0.642 0.555 0.586 0.568 0.600
0.533 0.520 0.468 0.595 0.697 0.651 0.431 0.458 0.500 0.636 0.600 0.617
0.636 0.521 0.604 0.651 0.434 0.651 0.700 0.675 0.702 0.642 0.642 0.702
0.789 0.700 0.783 0.705 0.764 0.742 1.000
0.326 0.479 0.380 0.591 0.460 0.608 0.645 0.704 0.583 0.612 0.562 0.625
0.530 0.519 0.470 0.723 0.795 0.673 0.437 0.551 0.666 0.659 0.659 0.782
0.695 0.551 0.666 0.604 0.469 0.711 0.720 0.659 0.642 0.595 0.630 0.707
0.761 0.682 0.800 0.600 0.609 0.634 0.707 1.000
0.261 0.369 0.244 0.521 0.326 0.571 0.577 0.675 0.478 0.543 0.488 0.521
0.456 0.448 0.488 0.586 0.651 0.682 0.326 0.446 0.456 0.627 0.590 0.608
0.627 0.545 0.634 0.682 0.523 0.604 0.609 0.666 0.741 0.595 0.595 0.675
0.736 0.605 0.729 0.513 0.567 0.594 0.631 0.619 1.000
0.395 0.428 0.387 0.673 0.500 0.738 0.733 0.720 0.630 0.695 0.644 0.673
0.644 0.591 0.541 0.632 0.733 0.767 0.511 0.595 0.574 0.750 0.673 0.723
0.750 0.630 0.644 0.727 0.543 0.767 0.690 0.674 0.609 0.761 0.720 0.602
0.780 0.658 0.703 0.702 0.756 0.738 0.769 0.630 0.634 1.000

0.361 0.480 0.440 0.653 0.461 0.673 0.708 0.659 0.549 0.607 0.560 0.588

0.529 0.576 0.529 0.647 0.744 0.702 0.440 0.580 0.625 0.653 0.620 0.708

0.653 0.549 0.659 0.600 0.530 0.702 0.638 0.583 0.531 0.625 0.659 0.659

0.750 0.600 0.744 0.558 0.642 0.666 0.622 0.717 0.577 0.772 1.000

0.358 0.500 0.452 0.489 0.413 0.500 0.510 0.558 0.511 0.577 0.488 0.555

0.456 0.510 0.488 0.489 0.613 0.568 0.418 0.545 0.425 0.555 0.521 0.574

0.555 0.478 0.488 0.500 0.454 0.568 0.571 0.444 0.418 0.558 0.522 0.589

0.500 0.452 0.488 0.435 0.567 0.475 0.512 0.478 0.434 0.595 0.651 1.000

0.485 0.550 0.500 0.533 0.560 0.547 0.489 0.534 0.558 0.590 0.534 0.604

0.500 0.489 0.466 0.500 0.627 0.545 0.538 0.522 0.500 0.568 0.533 0.553

0.533 0.488 0.500 0.511 0.431 0.545 0.710 0.523 0.500 0.650 0.571 0.648

0.547 0.666 0.536 0.527 0.628 0.487 0.648 0.558 0.404 0.609 0.555 0.685

1.000

0.432 0.500 0.452 0.590 0.625 0.609 0.543 0.634 0.581 0.651 0.558 0.627

0.522 0.543 0.488 0.489 0.613 0.642 0.525 0.581 0.522 0.627 0.555 0.574

0.555 0.511 0.595 0.533 0.454 0.533 0.692 0.511 0.525 0.634 0.522 0.631

0.534 0.605 0.488 0.513 0.567 0.439 0.512 0.511 0.500 0.595 0.543 0.621

0.787 0.1000

0.394 0.500 0.452 0.489 0.511 0.571 0.577 0.634 0.619 0.690 0.595 0.666

0.558 0.543 0.456 0.520 0.613 0.604 0.487 0.619 0.488 0.666 0.590 0.608

0.590 0.545 0.595 0.568 0.391 0.568 0.692 0.547 0.525 0.634 0.558 0.675

0.609 0.564 0.560 0.555 0.611 0.512 0.631 0.545 0.500 0.634 0.577 0.714

0.787 0.764 1.000

0.473 0.466 0.600 0.553 0.581 0.642 0.608 0.489 0.613 0.681 0.627 0.659

0.666 0.608 0.590 0.551 0.510 0.531 0.641 0.510 0.555 0.586 0.553 0.571

0.553 0.577 0.458 0.531 0.558 0.531 0.568 0.545 0.422 0.590 0.666 0.511

0.533 0.569 0.488 0.439 0.525 0.476 0.477 0.448 0.400 0.590 0.510 0.431

0.550 0.536 0.536 1.000

0.487 0.543 0.468 0.625 0.586 0.608 0.755 0.630 0.767 0.795 0.785 0.813

0.785 0.717 0.630 0.687 0.680 0.638 0.604 0.583 0.595 0.695 0.625 0.708

0.659 0.727 0.562 0.638 0.531 0.638 0.681 0.697 0.568 0.744 0.785 0.590

0.644 0.642 0.674 0.641 0.692 0.595 0.627 0.617 0.511 0.704 0.645 0.545

0.634 0.581 0.619 0.731 1.000

0.386 0.479 0.408 0.733 0.553 0.681 0.755 0.704 0.688 0.755 0.704 0.733

0.666 0.645 0.595 0.653 0.680 0.711 0.533 0.652 0.630 0.813 0.659 0.744

0.772 0.617 0.666 0.673 0.469 0.638 0.644 0.659 0.642 0.704 0.744 0.666

0.720 0.604 0.674 0.641 0.650 0.595 0.666 0.617 0.619 0.785 0.680 0.545

0.595 0.658 0.700 0.613 0.767 1.000

0.414 0.446 0.375 0.630 0.458 0.651 0.652 0.674 0.586 0.652 0.600 0.630

0.600 0.617 0.531 0.591 0.727 0.720 0.466 0.520 0.531 0.704 0.630 0.680

0.666 0.553 0.714 0.681 0.437 0.681 0.775 0.707 0.692 0.714 0.756 0.810

0.775 0.692 0.725 0.648 0.750 0.729 0.763 0.697 0.625 0.800 0.727 0.585

0.684 0.666 0.666 0.619 0.738 0.780 1.000

0.386 0.448 0.408 0.695 0.520 0.720 0.717 0.785 0.688 0.755 0.666 0.733

0.630 0.612 0.562 0.687 0.795 0.750 0.500 0.617 0.630 0.813 0.659 0.782

0.772 0.652 0.704 0.750 0.500 0.750 0.761 0.697 0.642 0.744 0.744 0.750

0.761 0.642 0.714 0.641 0.736 0.717 0.707 0.688 0.619 0.875 0.795 0.700

0.717 0.700 0.743 0.613 0.727 0.809 0.871 1.000

0.414 0.446 0.404 0.704 0.521 0.613 0.727 0.714 0.738 0.767 0.714 0.744

0.714 0.688 0.565 0.659 0.652 0.644 0.534 0.659 0.674 0.829 0.562 0.755

0.744 0.659 0.636 0.644 0.380 0.644 0.613 0.590 0.534 0.600 0.674 0.505

0.613 0.500 0.508 0.605 0.575 0.601 0.558 0.586 0.477 0.714 0.652 0.547

0.560 0.585 0.625 0.581 0.697 0.780 0.750 0.825 1.000

0.340 0.431 0.392 0.632 0.470 0.617 0.620 0.673 0.529 0.588 0.509 0.568

0.509 0.557 0.480 0.596 0.653 0.645 0.365 0.560 0.571 0.632 0.568 0.680

0.600 0.560 0.673 0.645 0.480 0.658 0.617 0.530 0.516 0.571 0.571 0.539

0.617 0.479 0.574 0.534 0.513 0.568 0.565 0.591 0.555 0.711 0.760 0.555

0.468 0.555 0.521 0.431 0.529 0.591 0.630 0.695 0.595 1.000

0.325 0.456 0.413 0.510 0.468 0.627 0.630 0.651 0.565 0.595 0.543 0.608

0.543 0.530 0.448 0.540 0.666 0.659 0.477 0.600 0.434 0.644 0.574 0.625

0.644 0.500 0.577 0.586 0.545 0.697 0.555 0.533 0.477 0.651 0.543 0.571

0.555 0.477 0.581 0.500 0.512 0.575 0.571 0.531 0.523 0.690 0.630 0.600

0.500 0.560 0.560 0.395 0.500 0.565 0.533 0.636 0.533 0.681 1.000

0.384 0.422 0.377 0.543 0.571 0.595 0.600 0.619 0.725 0.674 0.700 0.690

0.700 0.600 0.511 0.541 0.636 0.627 0.589 0.682 0.545 0.651 0.510 0.595

0.613 0.642 0.545 0.627 0.477 0.666 0.634 0.571 0.512 0.619 0.581 0.500

0.595 0.589 0.585 0.781 0.685 0.666 0.615 0.533 0.452 0.789 0.600 0.525

0.578 0.564 0.605 0.488 0.642 0.642 0.609 0.725 0.692 0.613 0.625 1.000

4.3.4 ***Grouping of genotypes based on molecular markers*** A set of 60 germplasm lines including three checks (precoz, shehore 74-3, L-4076) were subjected to similarity analysis based on molecular markers, they could be grouped into ten clusters (Table 4.21). Clusters VI had the maximum lines (12), followed by the clusters VIII (9), IV (8), IX (7), III (5) clusters I, II and X (4 each) V clusters had 2 genotypes.

Table 4.21 Clustering of 60-germplasm accession based on RAPD analysis.

Cluster	Number of Accessions	Name of germplasm Accession
I	4	L5, L19, L20, L52.
II	4	L48, L49, L50, L51.
III	5	L40, L41, L42, L59 L60.
IV	8	L33, L36, L37, L38, L39, L43, L44, L45.
V	2	L6, L21.
VI	12	L17, L18, L30, L27, L31, L46, L47, L54, L55, L56, L57, L58.
VII	5	L23, L28, L32, L34, L35.
VIII	9	L9, L10, L11, L12, L13, L14, L15, L26, L53.
IX	7	L4, L7, L8, L16, L22, L24, L25.
X	4	L1, L2, L3, L29.

DISCUSSION

India has made significant progress in food grain production in the last three decades. The grain production and productivity has been realized though planned investment in research and developmental activities. However, the production of pulses lagged behind the actual requirement. These crops are important for sustaining of our cropping systems through biological nitrogen fixation in the soil and nutritional security of weaker sections of the society, specially the children and lactating women. Among pulses, lentil has special significance in nutritional security as it is used as dry grains, besides the feed and green fodder for animals. But the productivity of this pulse is considerably low. The main reasons for low productivity are low yield potential of the genotypes under cultivation on the one hand and their cultivation under poor management in marginal lands on the other. For improving the genetic yield potential, planned research through blending the conventional and biotechnological tools has to be carried out. Before launching the genetic up gradation programme, basic information on variability (both in terms of magnitude and kind), genetic divergence, heritability, genetic advance, correlation, direct and indirect effects of different characters on yield and genetic markers should be available with the breeders. Keeping these in view, the present investigation was carried out and the results obtained have been discussed below:

5.1 Morphological markers

On the basis of 14 distinct morphological characters viz. growth habit, plant pubescence, pigmentation tendril formation, leaf size, leaf shape, leaf colour, stipule size, peduncle length, flower colour, testa colour, testa pattern, seed shape and cotyledon colour, 100 germplasm

lines were classified. Out of 44 categories of above fourteen characters, these 100 germplasm fell in all forty-three categories. In the present investigation observed that the 70% genotypes were prostrate and 30% were erect; 59% genotypes were pubescent and 29% were non pubescent; 75% genotypes were pigmented and 25% were non pigmented, 75% genotypes were tendrilled and 25% were non tendriled; 47% genotypes leaf were medium, 32% genotypes leaf were narrow and 21% genotypes leaf were broad; 68% genotypes leaf were acute and 32% were oval; 43% leaf were light green 38% were green and 19% leaf were dark green; 83% genotypes stipule were short and 17% long stipule; 59% genotypes peduncle were short and 41% were long peduncle; 48% genotypes flower were purple, 26% light purple, 21% white and 5% dark purple; 37% testa were brown, 17% green, 15% gray, 13% buff, 12% tan, 5% black and 1% testa were yellow; 69% testa were mottled and 31% were non-mottled; 58% seeds were spherical, 40% flat, 1% globe, 1% oval seed shape; 53% orange cotyledon, 38% yellow, 87% green, 1% brown cotyledon colour out of 100 germplasm lines and varieties. This indicated here maximum number of genotypes were prostrate, pigmented, light green in colour, having short stipule, purple flower colour, brown testa mottled spherical in shape and orange in cotyledon colour . This suggests that these fourteen characters are not assorted independently as most of them are governed by the genes situated on the same chromosome. Being an autogamous crop there is no possibility of exchange of these characters through natural out crossing, also there appears to be restricted choice for the genotypes having these characters.

On the basis of qualitative analysis 100 germplasm lines formed 10 clusters. The cluster I had the maximum lines (29) and cluster VII had

minimum lines (2); followed by the cluster X (15), VIII (13), VI(11), V (9), III (7), IX (6), I and IV (4).

5.2 Variability

The properties of continuous variation are essential and basic to the theory of evolution and to the practice of plant and animal genetic improvement. Most of the characters are governed by polygenes which are highly sensitive to the environmental factors. Hence the study of quantitative traits, although based on the Mendalian principles, has a different approach. Since in a continuous variation it is not possible to classify the genotypes on the basis of their phenotypic expression, the help of statistics is sought in analyzing the phenotypic data on metric traits.

The success of selection programme solely depends upon the magnitude and kind of variability in the base population. In the present investigation, very high magnitude of genetic coefficient of variability was observed for 100 seed weight, followed by number of primary and secondary branches, plant height, pods per plant, grain yield. Rajput *et al.* (1989), El.Attar.Ah *et al.* (1996), Ismail *et al.*, (1994), Kumar *et al.* (1995), Rakesh (1999), Singh *et al.* (1999), also reported Chakraborty *et al.* high genetic variability for these characters. Since these characters are the most important yield components and each of them is positively associated with seed yield, the existence of high genetic variability in these traits is a bright sign for effecting improvement in yield through simple selection combining together the characters, high number of pods per plant and large number of primary and secondary branches in a single genotype though intensive crossing. However, bi parental mating in the early generation shall has ten the process of combining the high number of pods per plant and a large number of primary and secondary branches

and finally help in increasing the seed yield. Therefore, a large segregation population has to be raised in order to ensure the combination having high mean values of these traits based on genetic make up. Also, the segregating population should be raised under well-managed condition in order to reduce the environmental variability.

Days to maturity and days to flowering though were found to be the low magnitude of the genetic variability, thus suggesting limited scope for altering the mean values of these characters through selection either from germplasm or in segregation populations.

It is interesting to mention that a large number of characters were found to be associated with similar magnitude of genotypic and phenotypic variability. This suggests that stable improvement in these characters through selection is quite possible. Being autogamous crop there is every possibility of a large fraction of genetic variability being contributed by additive component.

5.3 Genetic divergence

Genetic divergence is the measure of genetic distance among the cultivars or germplasm lines. The divergence may be due to geographical barriers or any other reasons, which may hinder the crossing resulting in the formulation of distinct groups.

Actually, on the basis of worldwide collection, Vavilov (1949) demonstrated that the genetic variability in most agricultural crop is not uniformly distributed in different parts of the ~~worked~~ world. He identified few regions, which he called as centers of origin, and according to him such centers carried maximum diversity for a particular crop plant. The variability could be seen in the form of wide wealth of land races, wild relatives and cultivated plants. However, Vavilov himself did not make a

distinction between the centers of diversity and centers of origin. He believed that the center of maximum diversity was also the center of origin for a crop plant. The modern view, however, is that the two centers may be entirely different. According to the modern theory, the cultivated plants did originate suddenly and the whole process has been very slow, beginning with the domestication of the wild plants followed by introgression of new genes from the wild relatives as it began to move out from the original center of diversity. The most important fact emerging from this concept is that not all crops have changed equally following their initial domestication. Thus, the grain legumes in contrast to cereal crops still retain some of their wild characteristics. This is because they have received much less attention in the form of human management. This fact should be kept in to consideration while planning plant-breeding programme in grain legumes, including lentil.

A set of 100 germplasm lines and varieties drawn from the germplasm collection were grouped into ten clusters based on divergence analysis. The earlier workers (Chahota *et al.*, 1992; Rathi *et al.*, 1998; Solanki *et al.*, 2000; Singh *et al.*, 2001; and Jeena *et al.*, 2002) could find clusters ranging from 3 to 9 that too among a limited number of varieties/ germplasm lines. The wide variation in the number of clusters seen in the present investigation based on as many as 100 germplasm lines and those which could be observed by the above workers on the basis of limited number of lines may be due to the purpose of selection of the base population for the study. The earlier studies were not only limited to grouping of genotypes but also for studies where selection of the genotypes was mainly based on genetic diversity.

However, in the present studies the selection of 100 germplasm lines was mainly based on the variation in fourteen morphological traits,

which theoretically formed 44 categories and its range was 2 to 7 as per trait when the germplasm lines were actually distributed. When these 44 categories based on morphological traits were superimposed with 12 quantitative traits on which the clustering was made the group/clusters reduced to 10 only. It is interesting to indicate that, in general, almost entire constellations of germplasm lines of each of the 44 morphological categories could get in to the particular cluster.

When the size of clusters was accounted for, the largest was VIII having 21 lines and the smallest ones III and VII, each having 5 lines. The cluster VIII having largest number of lines also had highest intra-cluster divergence, suggesting maximum variability among the lines falling in this cluster. On the other hand, the cluster III had minimum number of genotypes and cluster I had lowest intra cluster divergence. Therefore, the cluster VII had maximum divergence from other nine clusters, thus the lines from these two clusters (VII and XI) may be used in the crossing programme with desirable lines from rest of the clusters to deriver useful segregants. Similarly the divergence between clusters (IV and VII) has been found quite high, excepting of cluster VIII, IX, II, V, VI with cluster VII.

When the mean values of different characters were compared across the clusters, it has been observed that the clusters VIII which has maximum intra-cluster divergence the cluster IV had high mean values of 3 characters out of 12 under study on the other had rest of clusters had low to medium mean values of the characters. A set of 100 germplasm lines including three checks (Precoz, Sehore 74-3, L 4076) were subjected to D^2 analysis based on metric traits. They could be grouped into ten clusters.These 100 genotypes were also subjected to morphological and qualitative traits analysis and grouped into ten clusters. The distribution of 100 lines based on D^2 analysis and qualitative trait analysis in ten clusters is given in (Table 5.1).

Table 5.1 Distribution of germplasm lines of lentil in ten clusters based on quantitative and qualitative markers.

Clusters	Quantitative markers		Qualitative markers		Lines common in both the classification
	No. of Lines	Lines	No. Of lines	Lines	
I	9	L63, L68, L70, L71, L75, L81, L82, L89, L91.	29	L1, L3, L4, L5, L9, L10, L12, L13, L14, L16, L18, L19, L22, L26, L29, L31, L51, L56, L57, L67, L68, L78, L81, L84, L90, L93, L94, L95, L99.	L68, L81.
II	15	L2, L29, L30, L31, L32, L40, L42, L47, L48, L49, L61, L62, L64, L90, L92.	4	L15, L59, L64, L80.	L64.
III	5	L55, L79, L80, L85, L86.	7	L8, L11, L17, L24, L28, L30, L79.	L79.
IV	14	L6, L12, L15, L24, L26, L34, L35, L36, L38, L45, L46, L50, L53, L59.	4	L23, L32, L70, L71.	
V	4	L65, L67, L74, L76.	9	L53, L69, L85, L86, L87, L88, L89, L91, L92.	
VI	7	L60, L69, L77, L83, L93, L94, L99.	11	L6, L7, L21, L25, L27, L42, L44, L55, L58, L60, L72.	L60.
VII	5	L72, L73, L87, L88, L100.	2	L77, L83.	
VIII	21	L1, L3, L4, L5, L8, L9, L10, L11, L13, L14, L16, L17, L18, L19, L22, L28, L41, L56, L57, L58, L97.	13	L2, L38, L39, L45, L61, L63, L65, L73, L74, L96, L97, L98, L100.	L97.
IX	13	L7, L20, L21, L27, L37, L39, L43, L44, L51, L54, L66, L78, L84.	6	L20, L41, L62, L75, L76, L82.	L20.
X	7	L23, L25, L33, L52, L95, L96, L98.	15	L33, L34, L35, L36, L37, L40, L43, L46, L47, L48, L49, L50, L52, L54, L66.	L52.

In 100 lines, which were common in two-classification viz. based on D^2 analysis and qualitative trait analysis, only 8 (L68, L81, L79, L97, L64, L60, L20, L52) were found to be common.

5.4 Heritability and genetic advance

Heritability is a useful parameter for comparing and improving the efficiency of selection methods. It also helps to partition the total phenotypic variation into hereditary and environmental components. The reduction of the environmental error and genotype × environment interaction component of the phenotypic variance can be accomplished by increasing the number of replications in the breeding experiments. Heritability relates to the genetic control of variation and not the extent to which the oval mean performance is determined by genes. There may be large number of genes, which control a character such as flowering date. But every plant may be having the same genotype for most of them and hence, there may not be any genetic variation due to these genes. For a breeder that lexis are important which do vary in the population, because the genotype at these loci responds to selection and, therefore, heritability refers to those loci. Genetic advance, though not an independent entity, has an added advantage over heritability where the character is to be improved through selection in segregating generations. However, both the heritability and genetic advance are important in selection breeding Programme. Genetic gain could be estimated where genotypic variance is high, irrespective of the magnitude of heritability estimates. Heritability values in broad sense ranged from 39.9% for biomass score to 89.9% for 100 weight. likewise genetic advance in percentage of mean ranged from 10.87% for days to maturity to 51.5% for plant height. High heritability has been observed for a large number of characters (100 seed weight,

number of seed per pod, number of flower per peduncle, plant height, days to maturity, days to flowering).

Very high heritability has been reported by earlier workers in lentil. For example 90.80% for grain yield and 29.02% for shelling percentage by Bhajan *et al.* (1987), for number of seeds per plant and seed yield per plant by Homdi *et al.* (2003). The findings on heritability estimates are in quite agreement with those of above workers. This suggests that there is ample scope for selection breeding in respect of lentil improvement.

Excepting for days to maturity, biomass score and harvest index, the genetic advance was found reasonably high. The above workers also reported medium to high estimates of genetic advance for various characters, this further suggests that sufficient gain could be attained while selecting the desired types in the segregation generations.

5.5 Correlation

The aims of correlation studies is mainly to know the suitability of various characters for indirect selection response for one or more traits which results in correlated response for several other traits and the pattern of variation will also be compared. However, the relative selection efficiencies of direct and indirect selection must be compared before indirect selection can be advocated.

In the present studies, days to flowering with days for maturity, plant height and number of flower per peduncle; days to maturity with plant height; plant height with number of seed per pod; number of primary branches with 100-seed weight; number of secondary branches with pods per plant; pods per plant with 100-seed weight; 100-seed weight with grain yield and biomass score; and grain yield with biomass score were positively correlated. However, only few character combinations

showed negative and high correlation. Murari *et al.* (1988), Esmail *et al.*, (1994), Chauhan et al., (2001) and Naji *et al.*, (2003) also found high and positive correlation of seed/pod yield with other characters.

A very interesting inference has been drawn from the present study that, seed yield per plant had high and positive association with number of pods per plant, biomass score and 100-seed weight. Therefore, instead of selecting for yield *per se* selection should be exercised for number of pods per plant, biomass score and 100-seed weight, which will help in improving the yield automatically.

5.6 Path coefficient

Path coefficient is simply a standardized partial regression coefficient and as such, measures the direct influence of one variable upon another and permits the separation of correlation coefficient in to components of direct and indirect effects. The use of this method requires cause and effect of situation among the variables and the experiment must assign direction in the caused system based upon the ground or experimental evidence.

In the present study, though the number of pods per plant, 100-seeds weight and biomass score had high and positive correlation with seed yield, but excepting pods per plant and biomass score other two characters (number of secondary branches and 100-seed weight) had negligible direct effect on yield. It is interesting to note that besides having positive correlation with seed yield, biomass score and harvest index had very high direct effect. Therefore, these two characters should be harnessed for improving yield directly. Also, by increasing the plant height, number of primary/secondary branches pods per plant number of seed per pod could be increased and ultimately the seed yield, as these

two characters had substantial direct effect on yield. On the other hand, the contribution of biomass score and harvest index had negative, but substantial indirect effects on seed yield via number of pods per plant.

Similarly, Nigem *et al.* (1990, Luthra *et al.* (1990), Rasul *et al.*, (1990) Observed positive correlation of pods per plant, 100-seed weight, harvest index with seed yield. They also found direct effect of pod per plant, 100-seed weight, biomass score and harvest index could be observed. Therefore, direct selection for these trait would help increasing the yield. However, these traits would not improve the number of pods per plant, as they had negative indirect effect.

5.7 RAPD/Molecular analysis

The geographic region, morphological variability, breeding behavior (mode of reproduction) and stage/generation of the material are some of the primary factors, which contribute to the level of genetic/molecular diversity available in particular taxa. In the present investigation, 60 germplasm lines representing wide genetic variability including released varieties available in lentil were analyzed through 10 different decamer random primers of OPA, OPB, OPC, OPN, OPC, OPU, OPV and OPW series procured from M/S Operon Technologies Inc., USA. A very high level of polymorphism has been observed with these RAPD markers, revealing a wide and diverse genetic base of the germplasm accessions analyzed. The extremely low level of out crossing rules out the possibility of higher genetic diversity contributed by heterozygosity. In other words, the wide genetic diversity revealed by the RAPD analysis in the germplasm material studied in the present investigation is mainly because of inherent genetic differences at DNA level. A wide range of variability for morpho-agronomic traits including

protein patterns has also been reported by Tahir *et al.*, (1993) in lentil. Similar trends were also reported by Weeden *et al.*, (1994) while studying the phylogenetic analysis of lentil based on based on morphological (qualitative and quantitative character), biochemical and molecular RAPD markers. These workers suggested that RAPDs are abundant and reflect largely unselected genetic alterations. They provide a useful complement to most traditional systematic characters. There are many reports on use of RAPD markers for germplasm characterization in cotton, rice, and coffee and the results have revealed that despite the high level of polymorphism, these species have narrower genetic base (Iqbal *et al.*, 1997; Martin *et al.*, 1997; Lasshermes *et al.*, (1993). In these studies, the unique bands and profiles have been pointed out for one or a few accessions. On the contrary, in the present investigation on lentil 60 lines which also has a narrow genetic base, the common unique bands/profiles have been pointed out for most of the accessions (as high as 59 out of 60 accessions) and like variable polymorphic bands, the unique bands also hold promise for linking the genotypes in the lentil germplasm. As is evident from the results, it was not possible to identify a single common primer that could distinguish between all the lentil accessions. Similar results on lack of a single common primer that could act as a distinguishing tag in the germplasm have been reported by Lasshermes *et al.*, (1993) in coffee and Iqbal *et al.*, (1997) in cotton and the present results on lentil are in conformity with these investigation. The most polymorphic primers namely, OPB8, OPB18, OPU2 OPU14, OPV12 and OPW13 can effectively be used in DNA fingerprinting of lentil germplasm. In addition, these random primers may be helpful in genome mapping of lentil. These primers can also be used as probes to identify homologous sequences in other germplasm accessions.

It is interesting to note that the several genotypes specific band could be identified in the present investigation. Some of these unique band confined only to Sehore 74-3, L4378, L435, L4605, PL406, L4076, MC1, P22107, L5258, PKVL1, P22115, 10-3Y-26G germplasm lines; these can serve as a reference band for identification of the genotypes from large germplasm and their further detailed characterization.

A wide variation in different agro-morphological traits in the germplasm studied further substantiates the high degree of diversity observed at DNA/molecular level.

In 60 lines, which were common in two classification viz. based on D^2 analysis and similarity index, only 10 (L48, L49, L36, L38, L45, L9, L10, L11, L13, L14) were found common. On the other hand, when compared between morphological analysis and molecular analysis observed that only 5 accessions (L5, L19, L27, L55, L58) were found to be common (Tables 5.2, 5.3). This indicates distinct disparity in two systems of classification. The classification based on molecular markers appears to be more precise and accurate as the DNA content is not likely to be influenced by the environmental factors.

The molecular markers are most reliable for the characterization and classification of genotypes compared to that based on agro-morphological markers as the latter are influenced by the environmental factors during growth and developmental stages. In the present case, out of 60 genotypes, which were common in both the classification, ~~only 10 were found common.~~ These lines do not appear to have influence of the environment. Therefore, for having precise characterization of the genotypes, molecular markers should be used as a reliable aid.

Table 5.2 Distribution of germplasm lines of lentil in ten clusters based on qualitative analysis and molecular markers.

Clusters	Qualitative markers		Molecular markers		Lines common in both the classification
	No. of Line	Lines	No. of Line	Lines	
I	29	L1, L3, L4, L5, L9, L10, L12, L13, L14, L16, L18, L19, L22, L26, L29, L31, L51, L56, L57, L67, L68, L78, L81, L84, L90, L93, L94, L95, L99.	4	L5, L19, L20, L52.	L5, L19.
II	4	L15, L59, L64, L80.	4	L48, L49, L50, L51.	
III	7	L8, L11, L17, L24, L28, L30, L79.	5	L40, L41, L42, L59, L60.	
IV	4	L23, L32, L70, L71.	8	L33, L36, L37, L38, L39, L43, L44, L45.	
V	9	L53, L69, L85, L86, L87, L88, L89, L91, L92.	2	L6, L21.	
VI	11	L6, L7, L21, L25, L27, L42, L44, L55, L58, L60, L72.	12	L17, L18, L27, L30, L31, L46, L47, L54, L55, L56, L57, L58.	L27, L55, L58.
VII	2	L77, L83.	5	L23, L28, L32, L34, L35.	
VIII	13	L2, L38, L39, L45, L61, L63, L65, L73, L74, L96, L97, L98, L100.	9	L9, L10, L11, L12, L13, L14, L15, L26, L53.	
IX	6	L20, L41, L62, L75, L76, L82.	7	L4, L7, L8, L16, L22, L24, L25.	
X	15	L33, L34, L35, L36, L37, L40, L43, L46, L47, L48, L49, L50, L52, L54, L66.	4	L1, L2, L3, L29.	

Table 5.3 Distribution of germplasm lines of lentil in ten clusters based on agro-morphological and molecular markers.

Clusters	Agro-morphological markers		Molecular markers		Lines common in both the classification
	No. of lines	Lines	No. of lines	Lines	
I	9	L63, L68, L70, L71, L75, L81, L82, L89, L91.	4	L5, L19, L20, L52.	L48, L49.
II	15	L2, L29, L30, L31, L32, L40, L42, L47, L48, L49, L61, L62, L64, L90, L92.	4	L48, L49, L50, L51.	
III	5	L55, L79, L80, L85, L86.	5	L40, L41, L42, L59, L60.	
IV	14	L6, L12, L15, L24, L26, L34, L35, L36, L38, L45, L46, L50, L53, L59.	8	L33, L36, L37, L38, L39, L43, L44, L45.	L36, L38, L45.
V	4	L65, L67, L74, L76.	2	L6, L21.	
VI	7	L60, L69, L7, L83, L93, L94, L99.	12	L17, L18, L27, L30, L31, L46, L47, L54, L55, L56, L57, L58.	
VII	5	L72, L73, L87, L88, L100.	5	L23, L28, L32, L34, L35.	
VIII	21	L1, L3, L4, L5, L8, L9, L10, L11, L13, L14, L16, L17, L18, L19, L22, L28, L41, L56, L57, L58, L97.	9	L9, L10, L11, L12, L13, L44, L15, L26, L53.	L9, L10, L11, L13, L14.
IX	13	L7, L20, L21, L27, L37, L39, L43, L44, L51, L54, L66, L78, L84.	7	L4, L7, L8, L16, L22, L24, L25.	
X	7	L3, L25, L33, L52, L95, L96, L98.	4	L1, L2, L3, L29.	

SUMMARY

1 The present investigation **"Characterization of lentil germplasm through morphological and metric traits and molecular markers"** was under taken ~~together~~ following information on fourteen morphological and qualitative traits and twelve metric traits.

(1) Morphological markers/dendrogram.

(2) Analysis of variance.

(3) Mean, range and variability.

(4) Genetic divergence.

(5) Correlation.

(6) Path coefficient analysis.

(7) Heritability

(8) Genetic advance.

(9) Molecular markers/dendrogram.

2 The material used in the investigation comprised of 97 germplasm lines and varieties and 3 checks (Sehore 74-3, Precoz, L 4076) which were obtained form the division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi.

3 During 2003-2004, these 100 genotypes (97 germplasm lines and 3 checks) were raised in augmented randomized complete block design at the Research farm of IARI, New Delhi. Out of 100 germplasm lines grown during 2003-2004, 60 were selected on the basis of visual variability for molecular analysis. Again these 60 lines were raised in augmented randomized complete block design fashion for DNA extraction, PCR amplification and RAPD analysis.

4 During 2003-2004, four observations on randomly sampled plants of germplasm lines and checks were recorded on twelve metric traits (days to flowering and maturity, number of primary and secondary branch, plant height, number of flower per peduncle, pods per plant, seed per pod, 100-seed weight, grain yield biomass score, and harvest index).

5 Observations on several qualitative characters (growth habit, plant pubescence, pigmentation, tendril formation, leaf size, leaf shape leaf colour, stipule size, peduncle length, flower colour, testa colour, testa pattern, seed shape, cotyledon colour) were also recorded.

6 The leaf samples drawn from each of the 60 germplasm lines were used analyzed for the estimation of DNA profiles as per standard procedure.

7 Various statistical, biometrical and biotechnological techniques were followed for the analysis of the data on the above characters.

8 The results obtained on the above aspects are summarized as below.

9 The 100 germplasm lines and varieties and checks were distributed among 44 categories of 14 morphological and qualitative traits.

10 Out of 100 germplasm lines and varieties, this indicates here maximum number of genotypes were prostrate, pigmented leaf medium, oval and light green in colour having short peduncle and stipule, purple flower colour, brown testa-mottled, spherical in shape and orange cotyledon colour.

11 Out of 44 categories, 100 germplasm lines and checks were distributed all of categories among 14 character and as many as no single category remained un-represented.

12 On the basis of morphological and qualitative cluster analysis, 100 germplasm lines formed 10 clusters. The cluster I had the maximum lines (29) followed by the cluster X (15), VIII (13), VI (11), V (9), III (7), II and IV (4) and VII (2).

13 The variation each among 100 lines, including 3 checks, 97 un-replicated lines and 3 replicated checks was found highly significant, signifying genetic variability among the component lines of each group.

14 The analysis of variance of the data exhibited not significant variation due to replication, suggesting uniformity in the experimental field.

15 The 97 germplasm lines and 3 checks were compared and two groups were found genetically distinct.

16 Both genotypic and phenotypic coefficient of variability was found high for 100-seed weight, plant height, and number of secondary and primary branches in that order.

17 The magnitude of genotypic and phenotypic coefficient of variation was found almost identical in respect of pods per plant, grain yield, biomass score, number of flower per peduncle, harvest index and number of seed per pod.

18 The characters, days to flowering and days to maturity exhibited very low coefficient of variability. On the other hand, rest of the

characters were associated with medium to high values of this parameter.

19 On the basis of genetic divergence, 100 germplasm lines formed 10 clusters. The VIII have the maximum lines (21), followed by the clusters, II (15), IV (14), IX (13), I (9), X (7), III and VII (5) and V (4).

20 The intra-cluster divergence was found high for the cluster VII, VIII, X, medium for cluster II, III, V, IV, V, IX and low for I.

21 The divergence was found maximum between the VII and X moderate between III and IV, III and V, III and VI, III and VII, III and VIII, III and IX, III and X, II and VI, and low between rest of the cluster combinations;

22 A wide range in mean values of 12 characters across the clusters was recorded.

23 The cluster IV had high mean values of 3 characters out of 12 under study. On the other hand, rest of clusters had low to medium mean values of the characters.

24 The two system of classification that is based on D^2 analysis and qualitative analysis were found different as only 8 genotypes were found common.

25 A wide range in the estimates of heritability in broad sense was recorded among the characters.

26 The highest and lowest was exhibited by 100-seed weight and biomass score, respectively.

27 The characters days to flowering, days to maturity, plant height, number of flower per peduncle, seed per pod and 100 seed weight exhibited more than 80% heritability.

28 For the genetic advance, a wide range in the estimates from 10.60% for biomass score to 51.5% for plant height has been observed.

29 The relationship in the magnitude of the estimates of heritability and genetic advance has not shown any specific trend.

30 High and positive phenotypic correlation to days to flowering with days to maturity, plant height and number of flower per peduncle; day to maturity with plant height with number of ed per pod; number of primary branches with 100-seed weight; number of secondary branches with pods per plant; number of flower per peduncle with harvest index; pods per plant with 100-seed weight, grain yield and biomass score, seed per pod with yield and 100-seed weight with grain yield and harvest index were observed.

31 High and negative association was recorded between few character combinations (days to flowering with 100-seed weight; plant height with number of secondary branches; number of flower per peduncle with number of seed per pod; biomass score with harvest index).

32 Seed yield per plant being highly and positively associated with number of pods per plant, 100-seed weight and biomass score, the improvement in yield is expected through simple selection of the above component characters.

33 Out of 11 characters, 9 (days to flowering, days to maturity, plant height, number of secondary branches, pods per plant, seed per pod, 100-seed weight, biomass score, harvest index) had positive direct effect on seed yield. However, the biomass score had very high direct contribution.

34 Number of primary branches had flowering peduncle had indirect marginal effect on seed yield. However, between these two characters, number of primary branches had high negative direct effect.

35 Days to flowering via days to maturity, plant height, number of secondary branches, pods per plant, harvest index; days to maturity via day to flowering, plant height, number of secondary branches, pods per plant, seed per pod biomass score harvest index; plant height via days to flowering and maturity, number of primary branches, seed per pod , harvest index; number of primary branches via days to flower, number of secondary branches, day to maturity ; flower per peduncle, pods per plant, seed perpod,100-seed wt biomass score; number of secondary branches via days, flowering, day to maturity, flower per peduncle, pods per plant, seed per pod, days to flowering, biomass score; flower per peduncle via harvest maturity index; pods per plant via day to flowering and maturity, plant height, biomass score; seed per pod via day to maturity, plant height biomass score; number of secondary branches, flower per peduncle, pods per plant;

36 100-seed weight via flower per peduncle, pods per plant, biomass score; biomass score via days to maturity, plant height, number of secondary branches, flower per peduncle, pods per plant, seed per

pod, 100-seed weight; harvest index via days to flowering, days to maturity, number of primary branches, harvest index had positive indirect effect on seed yield.

Days to flowering via number of primary branches, flower per peduncle, seed per pod, 100-seed weight, biomass score; days to maturity via number of primary branches, flower per peduncle, 100-seed wt, plant height via number of secondary branches, flower per peduncle, pods per plant, 100-seed wt, biomass score; number of primary branches via plant height, harvest index; number of secondary branches via plant height, number of primary branches, 100-seed weight, harvest index; flower per peduncle via plant height, number of secondary branches, pods per plant, seed per pod 100-seed wt, biomass score. Pods per plant via number of primary branches, harvest index; 100-seed weight via days to flowering, days to maturity, plant height, number of primary branches, number of secondary branches, seed per pod, harvest index; harvest index via plant height, number of secondary branches, flower per peduncle, pods per plant, seed per pod, 100-seed weight and biomass score contributed negatively to seed yield.

For RAPD analysis of 60 genotypes, out of varying concentrations of template DNA 25g gave maximum number of reproducible bands and thus were considered most ideal.

Out of 200 primers, 100 were found sub-optimal or indistinct, inconsistent, non polymorphic, and thus, were discarded.

Among 20 primers, the amplification obtained from 10 primers (OPA1, OPB7, OPC4, OPN4, OPV6, OPV14, OPW3, OPW8, OPW9, OPW15) were inconsistent in all the 60 samples.

41 The number of fragments ranged from 2 to 10 and the size from 100 bp to 1.6 kb.

42 The selected primers gave a total of 44 amplification products, out of which 42 were found polymorphic.

43 On the basis of molecular markers 60 genotypes were grouped in to 10 clusters.

44 The three systems of classification that is based on agro-morphological- molecular and qualitative-molecular markers were found different as only 1 and 5 genotypes respectively were found common.

45 As compared to agro-morphological and qualitative markers, the molecular markers are independent of age, physiological status, tissues and environmental influences and relatively quick and easy to use, these can be used as distinguishing tags for characterizing the genetic diversity in lentil.

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